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Date: December 15, 1999
Docket No.: 1781-180P

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Transmitted herewith for filing is a patent application claiming priority under 35 U.S.C. § 119(e) of Provisional Application No. 60/112,499 filed on December 16, 1998.

Inventor(s): CHAN, Lily; CHUNG, Maxey Ching Ming; LIM, Renee Lay Hong

For: BACTERIAL-DERIVED MOLECULES AND THERAPEUTIC AND DIAGNOSTIC USES THEREFOR

Enclosed are:

- ☑ A specification consisting of Fifty-Six (56) pages
- ☑ Nine (9) sheet(s) of formal drawings
- ☒ An assignment of the invention
- ☐ Certified copy of Priority Document(s)
- ☑ Executed Declaration (☒ Original ☒ Photocopy)
- ☐ A statement (☐ original ☐ photocopy) to establish small entity status under 37 C.F.R. § 1.9 and 37 C.F.R. § 1.27
- ☑ Preliminary Amendment
- ☐ Information Disclosure Statement, PTO-1449 and reference(s)

☐ Other:

☒ Amend the specification by inserting before the first line the sentence:

--This application claims priority on provisional Application No. 60/112,499 filed on December 16, 1998, the entire contents of which are hereby incorporated by reference.--

The filing fee has been calculated as shown below:

			LARGE ENTITY	SMALL ENTITY
	BASIC FEE		**$760.00**	**$380.00**
	NUMBER FILED	NUMBER EXTRA	RATE FEE	RATE FEE
TOTAL CLAIMS	29- 20 =	9	X 18 = $162.00	X 9 = $0.00
INDEPENDENT CLAIMS	8- 3 =	5	x 78 = $390.00	X 39 = $0.00
☒ MULTIPLE DEPENDENT CLAIMS PRESENTED			+ $260.00	+ $130.00
		TOTAL	$1,572.00	$0.00

☑ A check in the amount of $1,612.00 to cover the filing fee and recording fee (if applicable) is enclosed.

☐ Please charge Deposit Account No. 02-2448 in the amount of $0.00. A triplicate copy of this transmittal form is enclosed.

☒ Please send correspondence to:

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If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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GMM/MAA/sas

Attachments

(Rev. 09/15/99)

PATENT
1781-180P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants: CHAN, Lily et al

Serial No.: New Group: Unknown

Filed: December 15, 1999 Examiner: Unknown

For: BACTERIAL-DERIVED MOLECULES AND THERAPEUTIC AND DIAGNOSTIC USES THEREFOR

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

December 15, 1999

Sir:

The following preliminary amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE CLAIM:

CLAIM 18: Line 1, change "any one of claims 1 to 9" to --claim 1--

CLAIM 22: Lines 1 and 2, change "any one of claims 1 to 9" to --claim 1--

PATENT
1781-180P

***** R E M A R K *****

The above amendment to the claims is merely to delete the improper multiple dependencies and place the application into better form prior to examination.

Favorable action on the above-identified application is respectfully requested.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §1.16 or under 37 C.F.R. §1.17; particularly, extension of time fees.

Respectfully submitted,

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BACTERIAL-DERIVED MOLECULES AND THERAPEUTIC AND DIAGNOSTIC USES THEREFOR

FIELD OF THE INVENTION

The present invention relates generally to molecules derived from a *Mycobacterium* species and recombinant, synthetic, derivative, homologue and analogue forms of said molecules. The molecules of the present invention are useful in diagnostic assays for *Mycobacterium* in biological and environmental samples. The present invention is particularly directed to molecules derived from *Mycobacterium tuberculosis* and related organisms and even more particularly to recombinant forms of these molecules or synthetic, derivative, homologue or analogue forms thereof and their use in diagnostic and therapeutic protocols for tuberculosis or other disease conditions associated with *M. tuberculosis* or related organisms.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

Bacterial infection represents a major cause of mortality and morbidity in human and other animal populations. One important group of bacteria are the mycobacteria. The mycobacteria are defined on the basis of a distinctive staining property conferred by their lipid-rich cell walls. The mycobacteria are relatively impermeable to various basic dyes but once stained, they retain dyes with tenacity. The mycobacteria have been referred to as "acid-fast" bacteria since they resist decolorization with acidified organic solvents (1). Mycobacteria range from widespread innocuous inhabitants of soil and water to organisms responsible for devastating and chronic diseases notably in tuberculosis and leprosy caused by *Mycobacterium tuberculosis* and *Mycobacterium leprae,* respectively.

Leprosy involves infection in skin tissue and can lead to disfigurement. Tuberculosis is generally confined to internal organs. Although both leprosy and tuberculosis were largely controlled by chemical intervention and improvements in living conditions, tuberculosis is now re-emerging as a major health problem. On an annual basis, reportedly between 2 and 3 million people die from tuberculosis, mostly in developing countries (2).

Conventional diagnostic tests for tuberculosis include chest X-ray, detecting the presence of acid-fast bacilli in clinical specimens and the skin test using tuberculin PPD (Purified Protein Derivative) [3]. However, these procedures are time intensive, frequently the results are ambiguous and X-ray machines are expensive and generally not portable enough for use in developing countries.

Nucleic acid probes can be used in a polymerase chain reaction (PCR) to specifically detect a mycobacterial infection, but require complex equipment, highly skilled staff and are too expensive for the developing countries (4). Rapid serological diagnostic tests are available on an ELISA or "strip" format which uses antigen(s) to detect antibody in sera (5). However, currently there is no satisfactory test for tuberculosis. A majority of *M. tuberculosis* antigens studied to date have homology with analogues proteins of other microorganisms that may or may not be pathogenic; resulting in cross-reactivity of these antigens to reactive serum antibodies in patients with inactive TB or nontuberculous infections (6). Hence, positive test results produced by these known antigens are generally unreliable and supplementary tests are required to confirm the presence of the tuberculosis infection.

In work leading up to the present invention, the inventors sought to use recombinant molecules from *M. tuberculosis* in the development of a highly specific and sensitive diagnostic test for tuberculosis. The same or similar molecules are also proposed for use as therapeutic agents for the treatment of tuberculosis.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The subject specification contains nucleotide and amino acid sequence information prepared using the programme PatentIn Version 2.1, presented herein after the bibliography. Each

nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc) and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide and amino acid sequences referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (eg. <400>1, <400>2, etc).

One aspect of the present invention provides an isolated polypeptide or a derivative, homologue, analogue or functional equivalent thereof wherein said polypeptide is obtainable from a species of *Mycobacterium* and which polypeptide is immunointeractive with sera from a human, animal or avian species exposed to said species of *Mycobacterium* or its relative or antigenic parts thereof but which polypeptide is substantially not immunointeractive with sera from a human, animal or avian species not prior exposed to said species of *Mycobacterium* or its relative or its antigenic parts.

Another aspect of the present invention is directed to an isolated polypeptide or a derivative, homologue, analogue or functional equivalent thereof wherein said polypeptide is obtainable from *M. tuberculosis* or a related organism and which polypeptide is immunointeractive with sera from a human previously exposed to *M. tuberculosis* or an antigenic extract therefrom but is substantially not immunointeractive with human sera not previously exposed to *M. tuberculosis* or a antigenic extract thereof.

Yet another aspect of the present invention relates to an isolated polypeptide obtainable from *M. tuberculosis* or related organism or a derivative, homologue, analogue or chemical equivalent of said polypeptide which polypeptide is immunointeractive with sera from a human patient with active pulmonary or extra-pulmonary tuberculosis but is substantially not immunointeractive with sera from a subject not previously infected with *M. tuberculosis* or sera from a subject who otherwise has no immunological memory for said polypeptide or antigenic derivatives thereof.

Still yet another aspect of the present invention provides a polypeptide having a molecular weight selected from about 5 kDa to about 100 kDa or a derivative, homologue, analogue or

functional equivalent thereof said polypeptide obtainable from *M. tuberculosis* and wherein polypeptide is immunointeractive with sera from a patient with active pulmonary or extra-pulmonary tuberculosis but substantially not immunointeractive with sera from a subject who does not have active pulmonary or extra-pulmonary tuberculosis.

In still yet another aspect of the present invention there is provided a polypeptide having a molecular weight selected from about 10 to 20 kDa, 28 to 38 kDa, 38 to 48 kDa, 53 to 63 kDa and 55 to 65 kDa or a derivative, homologue, analogue or functional equivalent thereof said polypeptide obtainable from *M. tuberculosis* and wherein polypeptide is immunointeractive with sera from a patient with active pulmonary or extra-pulmonary tuberculosis but substantially not immunointeractive with sera from a subject who does not have active pulmonary or extra-pulmonary tuberculosis.

Yet a further aspect of the present invention provides a polypeptide comprising an amino acid sequence selected from <400>2, <400>4, <400>6, <400>8, <400>10 or an amino acid sequence having at least 60% similarity to any one of said sequences.

Still yet a further aspect of the present invention provides a polypeptide encoded by a nucleotide sequence selected from <400>1, <400>3, <400>5, <400>7, <400>9 or an nucleotide sequence having at least 60% similarity to any one of said sequences or a nucleotide sequence capable of hybridizing to any one of said sequences under low stringency conditions at 42°C.

Another aspect of the present invention contemplates a method of isolating a polypeptide from *Mycobacterium* species said method comprising culturing cells of said *Mycobacterium* species in a growth medium to increase the number of cells to a sufficient population, harvesting said cells and subjecting said cells to protein extraction techniques to extract protein from said cells, fractionating the extracted protein and subjecting said protein to binding analysis with antibodies to said *Mycobacterium* species or antigenic portions thereof and isolating the polypeptides to which antibodies interact.

Yet another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a

polypeptide obtainable from a species of *Mycobacterium* and which polypeptide is immunointeractive with sera from a human, animal or avian species exposed to said species of *Mycobacterium* or its relative or antigenic parts thereof but which polypeptide is substantially not immunointeractive with sera from a human, animal or avian species not prior exposed to said species of *Mycobacterium* or its relative or its antigenic parts.

Another aspect of the present invention contemplates a method for detecting the presence of *M. tuberculosis* such as in a patient suffering from tuberculosis said method comprising contacting a biological sample from a patient or subject with an antibody specific for a polypeptide from said *M. tuberculosis* and detecting a complex between said polypeptide and said antibody.

Yet another aspect of the present invention provides a method for detecting the presence of *M. tuberculosis* such as in a patient suffering from tuberculosis said method comprising contacting a sera sample from a patient or subject with a polypeptide from *M. tuberculosis* and detecting a complex between said polypeptide and an antibody in said sera.

Still yet another aspect of the present invention provides an assay device for *M. tuberculosis* comprising a solid support having immobilized thereon one or more polypeptides obtainable from *M. tuberculosis* or derivatives, homologues, analogues or antigenic equivalents thereof and a portion of said solid support adapted for receiving a sample from a human subject to be tested wherein said sample would contain an antibody specific for said *M. tuberculosis* polypeptide if said subject has been exposed to *M. tuberculosis* wherein upon contact between the antibody from the subject and the immobilized polypeptide, a complex forms and said complex is detected by an anti-human immunoglobulin labelled with a reporter molecule.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation of the strategy for the isolation and expression of *M. tuberculosis* protein antigens.

Figure 2 is a photographic representation showing (A) gel purified and concentrated *M. tuberculosis* protein bands (B.1, 2, 3, 4, 5, 6, 8, 9, 10) blotted onto PVDF membrane and were then excised for N-terminal sequencing; (B) concentrated *M. tuberculosis* protein bands were

blotted onto nitrocellulose membrane and immuno-screened using pooled normal (N) and active (A) sera, respectively. Positive bands (arrows) were observed with A but not with N.

Figure 3 is a representation showing the results of homology search against the GenBank protein sequence databases. Proteins showing the highest homology to the *M. tuberculosis* protein bands are as shown.

Figure 4 is a photographical representation of Western blot screening of recombinant *M. tuberculosis* antigens. (A) Arrows indicate the position of the recombinant antigens on the membrane. M=Kaleidoscope protein marker and H=strip probed with anti-RGSHis, C= a positive control of strips probed with known human serum reactive to the specific recombinant antigen. (B) Reactivity is estimated based on the intensity of the band.

Figure 5 is a representation of the percentage of reactivity of recombinant TB antigens against different sera panels. A known 38kDa antigen (7, 8) of *M. tuberculosis* was included in the screening. The gene (GeneBank Accession # M30046) for this antigen was cloned, expressed in pQE30 and partially purified. Also shown are the percentages of reactivity of sera samples detected by a commercially available rapid TB diagnostic kit from ICT (Amrad).

Figure 6 is a graphical representation of a result from a Western screen using sera from normal individuals or patients with extrapulmonary tuberculosis, pulmonary tuberculosis and inactive tuberculosis.

Figure 7 is a graphical representation showing the percentage of reactivity for various combinations of these recombinant antigens against the inactive, active (pulmonary) and active (extra-pulmonary) sera panels respectively.

Figure 8 is a graphical representation showing the comparison of reactivity against the different sera panel for the combination of all the recombinant TB antigens compared to the ICT TB diagnostic kit. The graph shows that the percentage of reactivity for the active sera panel (pulmonary and extra-pulmonary) is higher than that observed for the kit.

Figure 9 is a graphical representation showing that the combination of antigens (B.6 + B.10 + MMP + 38 kDa) gave a sensitivity (60-70%) higher than that observed for the ICT kit (50%).

A summary of the sequence listing is shown below:

Sequence	**Sequence Identity No.**
Nucleotide sequence of antigen B.4	<400>1
Amino acid sequence of antigen B.4	<400>2
Nucleotide sequence of antigen B.6	<400>3
Amino acid sequence of antigen B.6	<400>4
Nucleotide sequence of antigen B.10	<400>5
Amino acid sequence of antigen B.10	<400>6
Nucleotide sequence of antigen MMP	<400>7
Amino acid sequence of antigen MMP	<400>8
Nucleotide sequence of antigen C17	<400>9
Amino acid sequence of antigen C17	<400>10

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification of molecules from *Mycobacterium* species which are immunointeractive with antigen-specific molecules from humans, animals or birds which have been infected with the *Mycobacterium* species or its relative or extracts thereof or following administration to humans, animals or birds the molecule itself or in combination with a mixture of molecules. Detection of the molecules from *Mycobacterium* species or antibodies thereto is indicative of the presence of the particular species of *Mycobacterium* and hence has both diagnostic and therapeutic implications.

Accordingly, one aspect of the present invention provides an isolated polypeptide or a derivative, homologue, analogue or functional equivalent thereof wherein said polypeptide is obtainable from a species of *Mycobacterium* and which polypeptide is immunointeractive with sera from a human, animal or avian species exposed to said species of *Mycobacterium* or its relative or antigenic parts thereof but which polypeptide is substantially not immunointeractive with sera from a human, animal or avian species not prior exposed to said species of *Mycobacterium* or its relative or its antigenic parts.

For the purposes of exemplifying the present invention, the preferred species of *Mycobacterium* is *M. tuberculosis.* However, the present invention also extends to its relatives, *Mycobacterium bovis* and *Mycobacterium africanum.* In addition, the present invention further contemplates other mycobacteria including but not limited to *Mycobacterium avium, Mycobacterium microti, Mycobacterium leprae, Mycobacterium lepraemurium, Mycobacteria paratuberculosis, Mycobacterium ulcerans, Mycobacterium marinum, Mycobacterium smegmatis, Mycobacterium intracellulare, Mycobacterium xenopi, Mycobacterium chelonei, Mycobacterium fortuitum, Mycobacterium farcinogenes, Mycobacterium flavum, Mycobacterium haemophitum, Mycobacterium kansasii, Mycobacterium phlei, Mycobacterium scrofulaceum, Mycobacterium senegalense, Mycobacterium simiae, Mycobacterium thermoresistible,* and *Mycobacterium xenopi.*
Accordingly, in a preferred embodiment, the present invention is directed to an isolated polypeptide or a derivative, homologue, analogue or functional equivalent thereof wherein

said polypeptide is obtainable from *M. tuberculosis* or a related organism and which polypeptide is immunointeractive with sera from a human previously exposed to *M. tuberculosis* or an antigenic extract therefrom but is substantially not immunointeractive with human sera not previously exposed to *M. tuberculosis* or a antigenic extract thereof.

In the comparison to human sera not previously exposed to *M. tuberculosis,* this does not exclude sera from subjects previously exposed to other species of *Mycobacterium* or other genera having biochemical or genetic but not epidemeological properties related to *M. tuberculosis.*

The sera preferably contains antibodies to the polypeptide of the present invention. The present invention extends, however, to other antigen-specific molecules or components of the immune system having antigen specificity including but not limited to cells carrying surface immunoglobulins specific to the polypeptide and T-cell derived antigen binding molecules (TABMs).

Preferably, the immunointeraction is an interaction between an antibody in the sera of a person previously exposed to *M. tuberculosis* or a related organism or an antigen containing extract therefrom and the polypeptide or its antigenic derivatives from *M. tuberculosis.* The term "related" in this context means another species of *Mycobacterium* or strain of *M. tuberculosis* which is associated with a similar disease as caused or exacerbated by *M. tuberculosis.*

Generally, a person "previously" exposed to *M. tuberculosis* or its antigen extract, is a person exhibiting immunological memory of the interaction and, hence, carrying antibodies in the sera to an antigen of *M. tuberculosis.* Conveniently, the sera are from patients with active pulmonary or extra-pulmonary tuberculosis.

As stated above, reference to a relative to *M. tuberculosis* includes various strains of *M. tuberculosis* as well as different species of *Mycobacterium* which contain the same polypeptide or an antigenically related polypeptide. Examples of related species include *M. bovis* and *M. africanium.* Such related strains or species would induce or be associated with similar disease conditions induced by *M. tuberculosis.*

Accordingly, another aspect of the present invention provides an isolated polypeptide

obtainable from *M. tuberculosis* or related organism or a derivative, homologue, analogue or chemical equivalent of said polypeptide which polypeptide is immunointeractive with sera from a human patient with active pulmonary or extra-pulmonary tuberculosis but is substantially not immunointeractive with sera from a subject not previously infected with *M. tuberculosis* or sera from a subject who otherwise has no immunological memory for said polypeptide or antigenic derivatives thereof.

In a preferred embodiment, the polypeptides of the present invention from *M. tuberculosis* range in molecular weight from about 5 kDa to about 100 kDa. More preferably, the polypeptides have a molecular weight range of from about 10 to 20 kDa, 28 to 38 kDa, 38 to 48 kDa, 53 to 63 kDa and 55 to 65 kDa.

In a most preferred embodiment, the molecular weight of the polypeptides are selected from 16±3, 33±3, 38±3, 55±3 and 56±3 kDa.

Accordingly, another aspect of the present invention is directed to a polypeptide having a molecular weight selected from about 5 kDa to about 100 kDa or a derivative, homologue, analogue or functional equivalent thereof said polypeptide obtainable from *M. tuberculosis* and wherein the polypeptide is immunointeractive with sera from a patient with active pulmonary or extra-pulmonary tuberculosis but substantially not immunointeractive with sera from a subject who does not have active pulmonary or extra-pulmonary tuberculosis.

More particularly, the present invention provides a polypeptide having a molecular weight selected from 16±3, 33±3, 38±3, 55±3 and 56±3 kDa or a derivative, homologue, analogue or functional equivalent thereof said polypeptide obtainable from *M. tuberculosis* and wherein polypeptide is immunointeractive with sera from a patient with active pulmonary or extra-pulmonary tuberculosis but substantially not immunointeractive with sera from a subject who does not have active pulmonary or extra-pulmonary tuberculosis.

The present invention is particularly exemplified in relation to *Mycobacterium* antigens B.4, B.6, B.10, MMP and C17 having amino acid sequences and corresponding nucleotide sequences as set forth in <400>1, <400>3, <400>5, <400>7, <400>9 respectively.

Accordingly, another aspect of the present invention provides a polypeptide comprising an amino acid sequence selected from <400>2, <400>4, <400>6, <400>8, <400>10 or an amino acid sequence having at least 60% similarity to any one of said sequences.

Yet a further aspect of the present invention provides a polypeptide encoded by a nucleotide sequence selected from <400>1, <400>3, <400>5, <400>7, <400>9 or an nucleotide sequence having at least 60% similarity to any one of said sequences or a nucleotide sequence capable of hybridizing to any one of said sequences under low stringency conditions at 42°C.

Even yet a further aspect of the present invention provides a nucleotide sequence selected from <400>1, <400>3, <400>5, <400>7, <400>9 or an nucleotide sequence having at least 60% similarity to any one of said sequences or a nucleotide sequence capable of hybridizing to any one of said sequences under low stringency conditions at 42°C.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps. Gap uses the alignment method of Needleman and Wunsch (19). Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website http://mel1.angis.org.au.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions.

Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41$ (G+C)% (17). However, the T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatch base pairs (18).

The present invention also provides genetic constructs comprising nucleic acid molecules encoding the *Mycobacterium* antigens as well as cells transformed with same. Examples of such cells include *Mycobacterium* cells, *E. coli,* insect cells, yeast cells, mammalian cells and plant cells.

The term "derivative" includes mutants, fragments and parts of the polypeptide of the present invention including single and multiple amino acid substitutions, additions and/or deletions to the naturally occurring amino acid sequences.

Preferably, the derivative is an antigenic fragment of the subject polypeptide meaning it contains an epitope required for an antibody in the sera of a patient with active pulmonary or extra-pulmonary tuberculosis to bind to the antigenic fragment.

A homologue includes functionally, structurally or stereochemically similar polypeptides from other species of *Mycobacterium* or other genera of bacteria. A homologue as contemplated herein includes a mimotope. The use of mimotopes is quite useful in competing with *Mycobacterium* polypeptide antigens for binding to antibodies. This competition may then be used to determine the concentration of polypeptides.

Analogues and mimetics include molecules which contain non-naturally occurring amino acids and which do not contain amino acids but nevertheless behave functionally the same as the polypeptide. Natural product screening is one useful strategy for identifying analogues

and mimetics. Natural product screening involves screening environments such as bacteria, plants, riverbeds, seabeds, aquatic environments, coral and antarctic or arctic environments for naturally occurring molecules which mimic, agonise or antagonise the function of the *Mycobacterium* polypeptide of the present invention.

Chemical libraries may also be screened for synthetic mimetics, agonists, antagonists or chemical analogues of the subject polypeptides.

Analogues of the subject peptides contemplated herein also include modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptide molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol

and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid contemplated herein is shown in Table 1.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu

	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
5	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
10	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
15	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
	D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
	D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
20	D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
	D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
	D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
	D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
25	D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
	D-α-methylleucine	Dmleu	α-napthylalanine	Anap
	D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
	D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
30	D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp

	D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
5	D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
10	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
15	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
20	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
25	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
	L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L-α-methylalanine	Mala
30	L-α-methylarginine	Marg	L-α-methylasparagine	Masn
	L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
	L-α-methylcysteine	Mcys	L-methylethylglycine	Metg

	L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
	L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
	L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
5	L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
	L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
	L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
	L-α-methylserine	Mser	L-α-methylthreonine	Mthr
	L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
10	L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
	N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
	1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

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Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide
20 and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C
25 termini, between two side chains or between a side chain and the N or C terminus.

All these types of modifications may also be important to stabilise the subject polypeptide if used in a diagnostic or therapeutic test.

The present invention further contemplates functional equivalents of the subject polypeptides. Functional equivalents may not necessarily be derived from the polypeptides

themselves but may share certain conformational similarities. Alternatively, functional equivalents may be specifically designed to mimic certain physiochemical properties of the polypeptides. Functional equivalents may be chemically synthesised or may be detected following, for example, natural product screening.

Reference to the subject polypeptide from *Mycobacterium* species should be read as including reference to all forms of the polypeptide including, by way of example, isoforms or monomeric, dimeric or multimeric forms or peptide fragments of the polypeptide as well as derivatives, homologues, analogues and functional equivalents thereof.

The polypeptide of the present invention may contain a range of other molecules fused, linked, bound or otherwise associated to the polypeptide such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins.

The polypeptides of the present invention may be a purified naturally occurring molecule, produced by chemical synthetic techniques or may be produced by recombinant DNA technology.

The present invention further contemplates a method of isolating a polypeptide from *Mycobacterium* species said method comprising culturing cells of said *Mycobacterium* species in a growth medium to increase the number of cells to a sufficient population, harvesting said cells and subjecting said cells to protein extraction techniques to extract protein from said cells, fractionating the extracted protein and subjecting said protein to binding analysis with antibodies to said *Mycobacterium* species or antigenic portions thereof and isolating the polypeptides to which antibodies interact.

Preferably, the *Mycobacterium* species in *M. tuberculosis.*

Preferably, the growth medium is Lowenstein-Jensen medium.

Preferably, fractionation of total polypeptides from *M. tuberculosis* is conducted by SDS-PAGE.

Preferably, the binding analysis in a Western blot analysis.

The isolated polypeptide bands are then generally subjected to N-terminal, C-terminal or internal amino acid sequencing. Nucleotide probes are then produced and cDNA or genomic libraries screened for genetic sequences encoding the isolated polypeptides.

In a particularly preferred embodiment, the polypeptide of the present invention comprises an N-terminal amino acid sequence substantially as set forth in one of SEQ ID NOs: 1 to 10 or an amino acid sequence having at least about 70% similarity thereto.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps. Gap uses the alignment method of Needleman and Wunsch (9). Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website http://mel1.angis.org.au..

Yet another aspect of the present invention is directed to genetic sequences encoding the polypeptide herein described.

Accordingly, another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a polypeptide obtainable from a species of *Mycobacterium* and which polypeptide is immunointeractive with sera from a human, animal or avian species exposed to said species of

Mycobacterium or its relative or antigenic parts thereof but which polypeptide is substantially not immunointeractive with sera from a human, animal or avian species not prior exposed to said species of *Mycobacterium* or its relative or its antigenic parts.

The preferred *Mycobacterium* species in *M. tuberculosis* although the present invention extends to any species of *Mycobacterium* or its relatives.

Preferably, the nucleotide sequence encodes an amino acid sequence substantially as set forth in one of SEQ ID NOs: 1 to 10 or an amino acid sequence having at least about 70% similarity thereto.

The nucleic acid molecule of this aspect of the present invention may be cDNA, genomic DNA or mRNA or cDNA/genomic DNA or DNA/RNA hybrids. The nucleotide sequence may encode the amino acid sequence of the naturally occurring polypeptide or it may encode a mutant, fragment, part or other derivative of the polypeptide. Accordingly, the nucleotide sequence may contain one or more nucleotide substitutions, deletions and/or additions to the naturally occurring sequence.

The nucleotide acid molecule of the present invention may be linear or covalently closed single or double stranded molecules, alone or as part of a genetic construct such as an expression vector and/or purification vector.

Still another aspect of the present invention is directed to antibodies to the subject polypeptides or their derivatives, homologues, analogues, mimetics and functional equivalents thereof. Such antibodies may be monoclonal or polyclonal. Where the derivatives are peptides, these may first need to be associated with a carrier molecule in order to induce antibody formation.

The antibodies of the present invention are particularly useful as therapeutic or diagnostic agents. For example, specific antibodies can be used to assist in screening for polypeptides in immunoassays or used as antagonists to inhibit polypeptide activity under certain circumstances. Techniques for such immunoassays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of polypeptide levels may be

important for monitoring certain therapeutic protocols.

As stated above, the antibodies may be monoclonal or polyclonal antibodies. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies.

As stated above, specific antibodies can be used to screen for the subject polypeptides. The latter would be important, for example, as a means for screening for levels of polypeptides in a cell extract or other biological fluid or purifying polypeptides made by recombinant means from culture supernatant fluid. The antibodies may also be used to screen for the presence of particular *Mycobacterium* polypeptides. The presence of *M. tuberculosis* polypeptides, for example, is indicative of tuberculosis.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of polypeptide.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the *Mycobacterium* polypeptides either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of polypeptide, or antigenic parts thereof, collecting serum from the animal and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The

preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting a subject polypeptide in a biological sample from a subject or culture supernatant flow or other source said method comprising contacting said biological sample with an antibody specific for said polypeptide or its derivative, homologue, analogue, mimetic or chemical equivalent thereof for a time and under conditions sufficient for an antibody-polypeptide complex to form, and then detecting said complex.

The presence of the polypeptide may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in one form of forward assay, an unlabelled *Mycobacterium* polypeptide is immobilized on a solid substrate and a sample of animal (e.g. human) sera to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of a polypeptide-antibody complex, a second antibody specific to animal (e.g. human) immunoglobulin, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of polypeptide-antibody labelled antibody. Any unreacted material is washed away, and the presence of polypeptide specific antibody determined by observation of a signal produced by the reporter molecule or the anti-animal (e.g. human) immunoglobulin. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added

simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. The method of this aspect of the present invention may readily be adapted for screening for *Mycobacterium* polypeptide by, for example, immobilizing an antibody specific for the polypeptide.

Immobilization of polypeptide or antibody either covalent or passive binding to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, nitrocellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, cover slips, slides or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing the polypeptide or antibody to the solid support. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to about 37°C) to allow binding of the polypeptide or antibody to its immobilized ligand. Following the incubation period, the solid phase is washed and dried and incubated with an antibody specific for a portion of the polypeptide or antibody. This antibody is linked to a reporter molecule which is used to indicate the binding of the antibody to its ligand.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or

radionuclide containing molecules (i.e. radioisotopes), chemiluminescent molecules colloidal material and precious metals such as gold.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, luciferase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the antibody-polypeptide complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-polypeptide-antibody. The substrate will react with the enzyme linked to the antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of polypeptide which is present in the sample. The reporter molecule also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the antibody-polypeptide complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the polypeptide of interest. Other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The polypeptides of the present invention and antibodies thereto are useful for detecting *Mycobacterium* infection and/or disease or other conditions associated with *Mycobacterium.* The presence of *Mycobacterium* in environmental samples may also be accomplished. In a particularly preferred embodiment, polypeptides derived from *M. tuberculosis* are detected. In this case, the polypeptides are particularly useful in detecting tuberculosis.

Accordingly, another aspect of the present invention contemplates a method for detecting the presence of *M. tuberculosis* such as in a patient suffering from tuberculosis said method comprising contacting a biological sample from a patient or subject with an antibody specific for a polypeptide from said *M. tuberculosis* and detecting a complex between said polypeptide and said antibody.

In an alternative embodiment there is provided a method for detecting the presence of *M. tuberculosis* such as in a patient suffering from tuberculosis said method comprising contacting a sera sample from a patient or subject with a polypeptide from *M. tuberculosis* and detecting a complex between said polypeptide and an antibody in said sera.

The polypeptide-antibody complex may be detected by any convenient means. One particularly useful method is to detect the complex using an anti-immunoglobulin labelled with a reporter molecule such as but not limited to colloidal gold, an enzyme, a radioactive isotope or a fluorescent compound.

In a particularly preferred embodiment, one or more polypeptides from *M. tuberculosis* are immobilized onto a solid support. Sera from a patient suspected of having exposure to *M. tuberculosis* are then brought into contact with immobilized polypeptides(s). After a time sufficient from an immobilized polypeptide-antibody complex to form, unbound material is washed away and an anti-human immunoglobulin labelled with a reporter molecule is brought into contact to bind to human antibodies in the sera of a patient which have bound to the immobilized *M. tuberculosis* polypeptide. The presence of an identifiable signal from the reporter molecule indicates the presence of the polypeptide and of *M. tuberculosis.* This is a particularly convenient means of identifying tuberculosis or a likelihood of tuberculosis or its possible development.

Reference herein to "tuberculosis" includes reference to pulmonary and extra-pulmonary

tuberculosis.

There are many different ways of conducting this assay. For example, an application matrix comprising absorable material such as filter paper and the like may comprise an application region, a *M. tuberculosis* polypeptide region and detection region. In the arrangement, sera from a patient or subject to be tested are applied to the application region and allowed to passage (i.e. "wick") along the application matrix through a region comprising impregnated *M. tuberculosis* polypeptide or its derivatives, homologues, analogues or antigenic equivalents. If antibodies are present in the sera specific for the polypeptide, a complex will form. This complex may be detected directly by the application of an anti-human immunoglobulin antibody labelled with a reporter molecule or the complex may be allowed to migrate into a third region impregnated with anti-human immunoglobulin antibody labelled with a reporter molecule. The identifiable signal produced by the reporter molecule can then be detected in that third region or in a fourth region where the sera-polypeptide-labelled antibody is permitted to concentrate.

In a particularly preferred embodiment, there is provided an assay device for *M. tuberculosis* comprising a solid support having immobilized thereon one or more polypeptides obtainable from *M. tuberculosis* or derivatives, homologues, analogues or antigenic equivalents thereof and a portion of said solid support adapted for receiving a sample from a human subject to be tested wherein said sample would contain an antibody specific for said *M. tuberculosis* polypeptide if said subject has been exposed to *M. tuberculosis* wherein upon contact between the antibody from the subject and the immobilized polypeptide, a complex forms and said complex is detected by an anti-human immunoglobulin labelled with a reporter molecule.

Yet another preferred assay technique comprises applying sera from a subject to be tested to an application matrix and allowing the sera to passage (i.e. "wick") along the matrix and into an area impregnated with recombinant polypeptides from *Mycobacterium* species and in particular *M. tuberculosis.* A complex forms between antibodies in the sera and the polypeptides and these complexes as well as free antibodies and free polypeptides continue to migrate to a region comprising anti-human antibodies labelled a reporter molecule such as, for example, gold. The passaging molecules then enter a region with reduced pore size such as an area containing nitrocellulose. The molecules which are not part of a complex pass through this region whereas complexes of antibodies and polypeptides tend to concentrate as a band in

front of the region with reduced pore size (e.g. nitrocellulose region). If the label is gold the line of complexes is pink or like colour or black. If silver stained or blue when the label is coloured latex particles.

Regardless of the assay procedure, and whether or not "wicking" is involved, one particularly useful procedure for detecting antibody-polypeptide interactivity is through electronic means such as described in US Patent No. 5,580,794.

All such electronic detection means are contemplated for use in accordance with the present invention. For example, interaction between certain molecules may lead to the production of an electrical signal which in turn, *via* a signal processor, correlates with the amount of interaction of amount of certain components of an interaction.

The present invention extends to the detection of a single type or species of *Mycobacterium* polypeptide as well as to the detection of a combination of *Mycobacterium* polypeptides. The detection of combinations of antigens may be accomplished, for example, by using a multiple array of antibodies.

The identification and isolation of the *Mycobacterium* species polypeptide of the present invention further permits the development of therapeutic protocols for generating, for example, an immune response directed to *Mycobacterium* species. Preferably, the *Mycobacerium* species is *M. tuberculosis.*

Accordingly, the present invention contemplates a composition comprising one or more polypeptides for *Mycobacterium* species or a derivative, homologue, analogue, mimetic or chemical equivalent thereof and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to as the "active ingredients". Preferably, the polypeptides are from *M. tuberculosis* and the composition is capable of inducing an immune response against *M. tuberculosis*.

The compositions suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. It must be stable under the conditions of manufacture and storage and must be

preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the polypeptide or other active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like.

The principal active ingredient, i.e. the polypeptide, will be present in the composition in an amount effective to induce an immune response. The composition may permit, for example, 0.01 μg to about 2000 mg/kg body weight.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating expression of genes encoding a polypeptide from *Mycobacterium* species. The vector may, for example, be a viral vector.

The present invention further contemplates the use of a polypeptide from *Mycobacterium* species such as *M. tuberculosis* or a derivative, homologue, analogue, mimetic or chemical equivalent thereof in the manufacture of a medicament for the treatment of *Mycobacerium* infection such as tuberculosis.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Extraction of *M. tuberculosis* total protein

Mycobacterium tuberculosis cells (ATCC 27294) were cultured in BBL MycoFlask containing Lowenstein-Jensen medium at 37°C with 10% v/v CO_2. After 2 weeks, the confluent cells were harvested, washed and resuspended in an equal volume of MilliQ H_2O. The cell suspension was heated to 90°C for 2h after which it was frozen at -20°C overnight. Thawed cells were sonicated for 2-3min and pelleted at 14,000rpm for 10min. Extraction of total protein was performed in 8M urea (equal ratio of weight of cells to volume of 8M urea). The cell suspension was vortexed at room temperature for 20 min heated at 90°C for 2min. Insoluble cellular debris was pelleted at 14,000rpm for 10min and the supernatant containing the extracted total protein was kept at -20°C until further use.

EXAMPLE 2

SDS-PAGE analysis and immunological analysis

The total protein extract from M. tuberculosis was fractionated on a 7.5% w/w SDS-PAGE (10) and transferred onto a nitrocellulose membrane by the conventional western blot method (11). Strips containing the Western blotted *M. tuberculosis* total proteins were immunoscreened with pooled sera from 9 patients having active tuberculosis (TB) and pooled sera from 7 normal non-infected individuals, respectively. Detection was carried out using the goat anti-human Ig conjugated with alkaline phosphatase (Harlan Sera lab,UK).

EXAMPLE 3

N-terminal sequencing of *M. tuberculosis* bands

Several individual protein bands (Mwt ranging from 10 to 160 kDa), which reacted positively in the immunoscreening, were excised from a preparative 7.5% w/v SDS-polyacrylamide gel, and concentrated on a long stacking gel (7cm of 4% w/v stacking gel, 5cm of 10% w/v resolving gel). After concentration, the protein bands were blotted onto a PVDF membrane and subsequently stained with Coomassie Brilliant Blue R-250. The stained protein bands were then excised from the membrane and used for N-terminal microsequencing. In addition, some of the protein bands were also blotted onto nitrocellulose membrane to repeat immunoscreening using the same pooled sera samples as previously described.

EXAMPLE 4

Screening the *Eco*R1 lambda ZAP phage expression library of M. tuberculosis genomic DNA

An expression library of *Eco*R1 restricted genomic DNA of *M. tuberculosis* was constructed in lambda ZAP Phage expression vector (according to the protocol by Stratagene (12)). The resultant library have 98% of recombinants ($2X10^6$pfu/μg arms) and insert sizes ranging from 0.7-2kb, observed in excised recombinant plasmids restricted with *Eco*R1 restriction enzyme. A lawn of XL1-MRF' cells infected with about $2x10^4$ pfu of the phage stock was prepared on a 150 mm plate, and incubated inverted, for 6-7 hr at 42°C. The lawn was then overlaid with a Hybond-C filters, presoaked in 1mM IPTG for induction of protein expression and were incubated at 37°C for 4hr, causing the transfer of expressed TB recombinant proteins from the plaques onto the membrane. The plate and filter were indexed for matching of corresponding plate and filter positions. About 20 plates were prepared for the library and as a negative control, a lawn of host cells infected with $2x10^4$ pfu of the non-recombinant lambda ZAP phage was used instead.

Filters were washed twice in TBST buffer (10mM Tris, ph7.5, 300mM NaCl, 0.005% v/v

Tween 20), blocked in 5% w/v skim milk in TBST, washed again in TBST and used to screen against 4 pooled active TB sera and pooled normal sera, respectively. The pre-absortion regime for the pooled sera was as follows: negative control filters containing proteins from induced lambda ZAP phage vector in the bacteria host was used. Pooled sera (diluted 1:100 in 1% w/v skim milk/TBST) was incubated (15ml/filter) for 2hr at RT after which sera was diluted to 1:200 in 1% w/v skim milk/TBST and transferred to a fresh control filter for overnight preabsorption. Screening was as follow: filters containing the expressed recombinant proteins from the phage library were incubated with pre-absorbed human sera for 2 hr, rocking at RT and washed three times in TBST. Secondary antibody of anti-human Ig - alkaline phosphatase conjugated (diluted 1:1000 in 1% w/v skim milk/TBST) was added and allowed to incubate for 1hr. After a final wash, the detection was by colormetric, using NBT/BCIP substrate. An immunoreactive recombinant clone, C17, was isolated and confirmed in the tertiary screening.

Secondary and tertiary plating: positive plaques were cored out and phage eluted in SM bufffer (13) containing 2% v/v chloroform and replated at about 100-200 pfu on a 82mm plates. The screening steps using pooled sera were repeated to confirm the positive clones. In the tertiary screening 6 positive phage recombinants were obtained and these were subsequently subjected to plasmid excision (12). Restriction enzyme *Eco*R1 digestion indicated that all the clones have a 2kb insert which was later confirmed to be identical by DNA sequencing. The clones were designated as C17 and have a 1.16 kb open reading frame (in frame with the vector ATG initiation codon) coding for a 38 ± 3 kDa protein.

EXAMPLE 5

Cloning and expression of genes for the *M. tuberculosis* antigens

Primers were designed from the N-terminal sequences. Polymerase chain reaction (PCR) was performed using the Advantage®-GC Genomic PCR kit (Clontech), *M. tuberculosis* genomic DNA (extracted as previously described (14)) and synthetic oligonucleotides. The PCR products were cloned into the pGEMT vector (Promega) before subcloning into the pQE30 expression vector (15) (Fig. 1). Expression was carried out in M_{15} *E.coli* cells, induced with 1mM IPTG. The M_{15} cells at 3hr after induction were harvested, and cell

pellet lysed in 8M urea buffer at pH6.5. The expressed recombinant protein contained a 6x histidine tag at the N-terminus which facilitated the purification with the Ni-NTA affinity column. Column washes were carried out in 8M urea buffer at pH 6.5 and pH 5.9 while subsequently elution of recombinant protein was carried out at pH4.5.

Table 2 provides the gene sizes for each TB antigen and the theoretical mass.

TABLE 2

GENE SIZE AND PHYSICAL CHARACTERISTICS OF TB ANTIGENS

Antigen	**Size of gene (kb)**	**Theoretical***	
		Molecular mass	**pI value**
B.4	1.617	55.8	5.12
B.6	1.560	55.0	5.03
B.10	0.903	32.9	4.95
MMP	0.432	16.1	5.00
C17	1.161	37.5	9.43

* obtained using the software "Compute pI/Mwt" from ExPASy homepage, Swiss Institute of Bioinformatics, Geneva

The gene size of each TB antigen and the theoretical molecular mass and pI values as calculated from the respective deduced amino acid sequence. The resultant recombinant proteins will be approximately 1.4 to 1.5-kDa larger than the theoretical molecular mass shown, due to the 6x Histidine tag at the N-terminal.

EXAMPLE 6

SDS-PAGE and Western blot of *M. tuberculosis* recombinant antigens

A total of 27μg of a partially purified recombinant antigen was subjected to a SDS-PAG electrophoresis at 180V for 1hr. The recombinant antigen was transferred from the polyacrylmide gel onto a Hybond-nitrocellulose membrane (Amersham) by Western blot (11), using the BioRad TransBlotter (according to manufacturer's protocol). After transfer, the membrane was blocked in 5% w/v milk/TBST, air dried and stored at 4°C until further use.

EXAMPLE 7

Screening of the recombinant *M. tuberculosis* antigens against active (pulmonary and extra-pulmonary), inactive and normal sera panels

Each membrane was cut into strips (a total of 23 strips can be obtained from each blot) and each strip was used for screening with a serum sample. One strip was used as an internal positive of known serum sample which reacted with the recombinant protein antigen and another was used to probe with the commercially available anti-RGSHis probe (QIAGEN). Sera samples were diluted to 1:100 in 1% w/v milk/TBST. Screening was carried out in tubes individually (2 strips/tube) and 3ml of diluted serum/tubes for 1hr with rocking at room temperature. The strips were then washed 3 times in TBST and were incubated with Goat anti-human Ig alkaline phosphatase conjugated (Harlan Sera lab) for 1hr with rocking at room temperature. The strips were washed 3 times in TBST and allowed to develop in 1ml of NBT/BCIP substrate (BioRad) for 4 mins.

Reactivity of recombinant protein to patient sera was interpreted based on the intensity of band observed, ie. negative, + and 2+; the later two were taken as a positive. Faintly stained bands were scored negative.

Using the above screening procedure, the five recombinant antigens were screened against a total of 85 human sera; 43 were from bacteriologically confirmed tuberculosis patients [23 pulmonary, 20 extra-pulmonary]; 22 inactive samples (with skin PPD tve but smear and

bacteria culture negative); and 20 sera samples of uninfected individuals previously vaccinated with BCG. All sera were stored at -70°C before use.

EXAMPLE 8

Identification of *M. tuberculosis* polypeptides

Immunological analysis of Western blotted *M. tuberculosis* total proteins gave 9 protein bands which reacted with the 9 pooled active sera but not with the 7 pooled normal sera. The respective bands were concentrated on a long stacking gel and excised. Excised protein bands were reactive with the pooled active sera but not with pooled normal sera, thus confirming the authenticity of these excised proteins as initially observed in the primary screening (Fig. 2). These proteins were identified by homology searches against protein sequence databases and the result gave a high percentage of homology to *Mycobacterium* proteins (Fig. 3). Primers for PCR were constructed to isolate gene that codes for proteins which gave the high homology (Fig. 1). B.4, B.6, B.10, C17 and MMP were among the genes in addition to B.5 (16), B.9 and 38 kDa isolated from *M. tuberculosis* genomic DNA.

The QIAGEN expression system was selected by the inventors for cloning and expression of the corresponding genes. This system utilizes the pQE30 vector whereby the expressed protein can be purified through a simple one step affinity chromatography and insoluble recombinant proteins can be purified under denaturation conditions (Fig. 1). Expression of recombinant proteins can be immuno-detected by the commercially available anti-RGSHis. In addition, the 6xHis tag is non-immunogenic and thus the purified recombinant protein can be used directly in a Western blot format for screening against the sera.

From the expression studies, B.4, B.5, B.9, MMP and 38 kDa gave high level of expression whereas B.6, B.10 and C17 expression level was low. All of these recombinant proteins were detected by anti-RGSHis. In addition, most of the recombinant proteins were insoluble except for B.5 and C 17 which was soluble. As such the format of choice for our initial screening was on a Western format.

Results of Western screening and the intensity of band observed on the strip is as shown in

Figure 4. Percentage of reactivity were calculated based on the number of sera which gave positive (+ or 2+ observed on the strip) divided by the number of sera sample screened. Figure 5 shows the percentage of reactivity of recombinant TB antigens against different sera panels. A known 38kDa antigen (7, 8) of *M. tuberculosis* was included in the screening. The gene (GeneBank Accession # M30046) for this antigen was cloned, expressed in pQE30 and partially purified. Also shown are the percentages of reactivity of sera samples detected by a commercially available rapid TB diagnostic kit from ICT (Amrad).

Analysis of the screening result is as shown in Figure 5 and Figure 6. B.6, B.9, B.10, C17, MMP and 38 kDa antigens showed no reactivity to the sera from uninfected individuals whereas B.4 and B.5 reacted to 5% and 25% of this panel respectively. B.4 and B.5 may have some epitopes which are recognised by antibodies present in uninfected animals. All of these recombinant antigens showed some reactivity to sera from the active TB panel, both pulmonary and extra-pulmonary. B.6 (52.2%) and B.10 (26.1%) seems to be specifically reactive to active pulmonary samples whereas MMP (25%) is specific to extra-pulmonary samples. All of the recombinant samples, except for B.5 and have low reactivity (<25%) to the inactive sera panel. This is important because a good seriological diagnostic marker would be one that allow differentiation of normal and individuals having previous infection of TB (inactive) from individuals with active tuberculosis.

Overall, the percentage of reactivity to sera panels increased when more recombinant antigens were included in the combination. This is in accordance with the observation that sensitivity increases with the number of antigens used for screening. Combinations including all recombinant antigens (B.4 + B.5 + B.6 + B.9 + B.10 + C17 + MMP+38kDa) gave a sensitivity of more than 90%. However the reactivity to inactive sera samples was also high (36%). Results indicate that the best combination of antigens to use is one that include B.6, B.10, B.4, MMP and C17 which gives a sensitivity of 60-70% to active sera, both pulmonary and extra-pulmonary. Specificity for this combination is 95% but reaches to 100% if excluding B.4 which as indicated earlier exhibited 5% reactivity to sera from uninfected individuals.

Comparison of reactivity against the different sera panel for the combination of all the

recombinant TB antigens compared to the ICT TB diagnostic kit. The graph shows that the percentage of reactivity for the active sera panel (pulmonary and extra-pulmonary) is higher than that observed for the kit.

Combination of antigens (B.6 + B.10 + MMP + 38 kDa) gave a sensitivity (60-70%) higher than that observed for the ICT kit (50%); the reactivity to inactive panel is comparable to that for ICT (Figure 9). As this is not an optimized assay, the sensitivity and specificity can be increased further using a panel of combined recombinant antigens which includes B.4, B.6, B.10, MMP and C17.

EXAMPLE 9

Determination of nucleotide and amino acid sequences of *Mycobacterium* antigens

Nucleotide sequences and corresponding amino acid sequences were determined for antigens B.4, B.6, B.10, C17 and MMP and are shown in <400>1, <400>3, <400>5, <400>7, <400>9 respectively.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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    290                 295                 300

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His Ala Leu Val Asp Pro Leu Val Gln Lys Pro Gly Lys Thr Ser Asp
305                 310                 315                 320

cca cgt ggc ctg agc tac gcc gac gcg acg acg ggc acc atc aac gcg  1008
Pro Arg Gly Leu Ser Tyr Ala Asp Ala Thr Thr Gly Thr Ile Asn Ala
                325                 330                 335

ctg tac agc cct cag cgc tgg aag tac ctg acc agt ggt ctg ctg ggg  1056
Leu Tyr Ser Pro Gln Arg Trp Lys Tyr Leu Thr Ser Gly Leu Leu Gly
            340                 345                 350

ctg cag cgc ggc agc gac gcc ggc gac ttg ctg gtg ctt gcc gac gac  1104
Leu Gln Arg Gly Ser Asp Ala Gly Asp Leu Leu Val Leu Ala Asp Asp
        355                 360                 365

tat gac ggc cgg gat gca gac ggg cac tac agc aac gac cag gac gcg  1152
Tyr Asp Gly Arg Asp Ala Asp Gly His Tyr Ser Asn Asp Gln Asp Ala
    370                 375                 380

ttc aac gcg gtc cgg tgc gtc tat gcg ccc aca ccg gcc gat cca gcg  1200
Phe Asn Ala Val Arg Cys Val Tyr Ala Pro Thr Pro Ala Asp Pro Ala
385                 390                 395                 400

gcc tgg gtg gcc gcc gac caa cgg atc cgt cag gtc gcc ccg ttc ctt  1248
Ala Trp Val Ala Ala Asp Gln Arg Ile Arg Gln Val Ala Pro Phe Leu
                405                 410                 415

agc tac ggg cag ttc acc gga tcc gcc ccc cgc gat ctg tgc gcg ctg  1296
Ser Tyr Gly Gln Phe Thr Gly Ser Ala Pro Arg Asp Leu Cys Ala Leu
            420                 425                 430

tgg ccg gtg ccg gca acg tcg acg ccg cac ccc gcg gcg ccg gcc ggg  1344
Trp Pro Val Pro Ala Thr Ser Thr Pro His Pro Ala Ala Pro Ala Gly
```

```
          435                 440                 445

gct ggc aag gtc gtc gtg gtg tcc acc acc cac gac ccg gcc act ccg   1392
Ala Gly Lys Val Val Val Val Ser Thr Thr His Asp Pro Ala Thr Pro
    450                 455                 460
tat cag tcc ggg gta gac ctg gcc cgc cag ctg ggc gca ccg ctg atc   1440
Tyr Gln Ser Gly Val Asp Leu Ala Arg Gln Leu Gly Ala Pro Leu Ile
465                 470                 475                 480

acc ttc gac ggc acc caa cac act gcg gtg ttc gat ggc aac cag tgt   1488
Thr Phe Asp Gly Thr Gln His Thr Ala Val Phe Asp Gly Asn Gln Cys
                485                 490                 495

gtg gac tct gcg gtg atg cac tat ttt ctc gac ggg acc ttg ccg ccg   1536
Val Asp Ser Ala Val Met His Tyr Phe Leu Asp Gly Thr Leu Pro Pro
            500                 505                 510

acg agt ctg cgg tgc gcg ccc tga                                   1560
Thr Ser Leu Arg Cys Ala Pro
        515                 520
```

<210> 4
<211> 519
<212> PRT
<213> Mycobacterium tuberculosis

<400> 4

```
Ala Ala Met Trp Arg Arg Arg Pro Leu Ser Ser Ala Leu Leu Ser Phe
  1               5                  10                  15
Gly Leu Leu Leu Gly Gly Leu Leu Leu Ala Ala Pro Pro Leu Ala Gly
            20                  25                  30
Ala Thr Glu Glu Pro Gly Ala Gly Gln Thr Pro Gly Ala Pro Val Val
        35                  40                  45
Ala Pro Gln Gln Ser Trp Asn Ser Cys Arg Glu Phe Ile Ala Asp Thr
    50                  55                  60
Ser Glu Ile Arg Thr Ala Arg Cys Ala Thr Val Ser Val Pro Val Asp
65                  70                  75                  80
Tyr Asp Gln Pro Gly Gly Thr Gln Ala Lys Leu Ala Val Ile Arg Val
                85                  90                  95
Pro Ala Thr Gly Gln Arg Phe Gly Ala Leu Leu Val Asn Pro Gly Gly
            100                 105                 110
Pro Gly Ala Ser Ala Val Asp Met Val Ala Ala Met Ala Pro Ala Ile
        115                 120                 125
Ala Asp Thr Asp Ile Leu Arg His Phe Asp Leu Val Gly Phe Asp Pro
    130                 135                 140
Arg Gly Val Gly His Ser Thr Pro Ala Leu Arg Cys Arg Thr Asp Ala
145                 150                 155                 160
Glu Phe Asp Ala Tyr Arg Arg Asp Pro Met Ala Asp Tyr Ser Pro Ala
                165                 170                 175
Gly Val Thr His Val Glu Gln Val Tyr Arg Gln Leu Ala Gln Asp Cys
            180                 185                 190
Val Asp Arg Met Gly Phe Ser Phe Leu Ala Asn Ile Gly Thr Ala Ser
        195                 200                 205
Val Ala Arg Asp Met Asp Met Val Arg Gln Ala Leu Gly Asp Asp Gln
    210                 215                 220
Ile Asn Tyr Leu Gly Tyr Ser Tyr Gly Thr Lys Leu Gly Thr Ala Tyr
225                 230                 235                 240
Leu Glu Arg Phe Gly Thr His Val Arg Ala Met Val Leu Asp Gly Ala
                245                 250                 255
Ile Asp Pro Ala Val Ser Pro Ile Glu Glu Ser Ile Ser Gln Met Ala
```

```
                260                 265                 270
Gly Phe Gln Thr Ala Phe Asn Asp Tyr Ala Ala Asp Cys Ala Arg Ser
        275                 280                 285
Pro Ala Cys Pro Leu Gly Thr Asp Ser Ala Gln Trp Val Asn Arg Tyr
    290                 295                 300
His Ala Leu Val Asp Pro Leu Val Gln Lys Pro Gly Lys Thr Ser Asp
305                 310                 315                 320
Pro Arg Gly Leu Ser Tyr Ala Asp Ala Thr Thr Gly Thr Ile Asn Ala
                325                 330                 335
Leu Tyr Ser Pro Gln Arg Trp Lys Tyr Leu Thr Ser Gly Leu Leu Gly
            340                 345                 350
Leu Gln Arg Gly Ser Asp Ala Gly Asp Leu Leu Val Leu Ala Asp Asp
        355                 360                 365
Tyr Asp Gly Arg Asp Ala Asp Gly His Tyr Ser Asn Asp Gln Asp Ala
    370                 375                 380
Phe Asn Ala Val Arg Cys Val Tyr Ala Pro Thr Pro Ala Asp Pro Ala
385                 390                 395                 400
Ala Trp Val Ala Ala Asp Gln Arg Ile Arg Gln Val Ala Pro Phe Leu
                405                 410                 415
Ser Tyr Gly Gln Phe Thr Gly Ser Ala Pro Arg Asp Leu Cys Ala Leu
            420                 425                 430
Trp Pro Val Pro Ala Thr Ser Thr Pro His Pro Ala Ala Pro Ala Gly
        435                 440                 445
Ala Gly Lys Val Val Val Val Ser Thr Thr His Asp Pro Ala Thr Pro
    450                 455                 460
Tyr Gln Ser Gly Val Asp Leu Ala Arg Gln Leu Gly Ala Pro Leu Ile
465                 470                 475                 480
Thr Phe Asp Gly Thr Gln His Thr Ala Val Phe Asp Gly Asn Gln Cys
                485                 490                 495
Val Asp Ser Ala Val Met His Tyr Phe Leu Asp Gly Thr Leu Pro Pro
            500                 505                 510
Thr Ser Leu Arg Cys Ala Pro
        515
```

<210> 5
<211> 903
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<221> CDS
<222> (1)..(903)

<220>
<221> mat_peptide
<222> (1)..(903)

<400> 5

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gat tac gaa cag gac tgg gac ggc gtt gcg ata acc ctg acg cgg tcg   48
Asp Tyr Glu Gln Asp Trp Asp Gly Val Ala Ile Thr Leu Thr Arg Ser
  1               5                  10                  15

cag ctg tat cgg cga acg ctg aat gtg gca cag gag ctg agc cgt tgt   96
Gln Leu Tyr Arg Arg Thr Leu Asn Val Ala Gln Glu Leu Ser Arg Cys
             20                  25                  30

ggt tcc acg ggt gac cgc gtg gtg atc tct gct ccg cag gga ctc gag   144
Gly Ser Thr Gly Asp Arg Val Val Ile Ser Ala Pro Gln Gly Leu Glu
         35                  40                  45
```

```
tac gtc gtc gcc tat ctc ggc gcg ttg cag gcc ggg cgc atc gcc gtg   192
Tyr Val Val Ala Tyr Leu Gly Ala Leu Gln Ala Gly Arg Ile Ala Val
     50                  55                  60

ccg ctt tcg gtt cca caa ggc ggc gtt acc gat gaa cgt tcc gat tcg   240
Pro Leu Ser Val Pro Gln Gly Gly Val Thr Asp Glu Arg Ser Asp Ser
 65                  70                  75                  80

gta ctg agt gat tcg tcg ccg gtg gcc att ctc act aca tcg tct gcc   288
Val Leu Ser Asp Ser Ser Pro Val Ala Ile Leu Thr Thr Ser Ser Ala
                85                  90                  95

gtg gac gac gtc gtg caa cat gtt gcg cgg cgg ccc ggg gaa tcc ccg   336
Val Asp Asp Val Val Gln His Val Ala Arg Arg Pro Gly Glu Ser Pro
            100                 105                 110

cca tca att atc gaa gtt gat ttg ctc gat ctg gac gct ccg aat ggg   384
Pro Ser Ile Ile Glu Val Asp Leu Leu Asp Leu Asp Ala Pro Asn Gly
        115                 120                 125

tat acc ttc aaa gaa gac gag tat cca tct acc gcg tat ttg caa tac   432
Tyr Thr Phe Lys Glu Asp Glu Tyr Pro Ser Thr Ala Tyr Leu Gln Tyr
    130                 135                 140

acc tcc ggg tcc acc cgc acg ccc gct ggc gtg gtg atg tcc cat cag   480
Thr Ser Gly Ser Thr Arg Thr Pro Ala Gly Val Val Met Ser His Gln
145                 150                 155                 160

aac gtt cgg gtt aat ttc gaa cag ctg atg tct ggc tac ttt gcg gat   528
Asn Val Arg Val Asn Phe Glu Gln Leu Met Ser Gly Tyr Phe Ala Asp
                165                 170                 175

acc gac ggg att cca ccg cca aat tcc gca ctc gta tcc tgg cta ccc   576
Thr Asp Gly Ile Pro Pro Pro Asn Ser Ala Leu Val Ser Trp Leu Pro
            180                 185                 190

ttc tac cac gac atg ggt ttg gta ata gga att tgc gca cca att ctg   624
Phe Tyr His Asp Met Gly Leu Val Ile Gly Ile Cys Ala Pro Ile Leu
        195                 200                 205

ggt gga tac ccc gcg gtg ctc acc agc ccg gtg tcg ttc ctg cag cgc   672
Gly Gly Tyr Pro Ala Val Leu Thr Ser Pro Val Ser Phe Leu Gln Arg
    210                 215                 220

ccg gcc cgg tgg atg cac ttg atg gcc agc gat ttt cac gcc ttt tcg   720
Pro Ala Arg Trp Met His Leu Met Ala Ser Asp Phe His Ala Phe Ser
225                 230                 235                 240

gca gca ccg aat ttc gcc ttt gaa cta gcg gca cga aga aca acc gac   768
Ala Ala Pro Asn Phe Ala Phe Glu Leu Ala Ala Arg Arg Thr Thr Asp
                245                 250                 255

gac gac atg gcc ggg cgt gac ctc ggc aac ata ctg acc atc ctc agc   816
Asp Asp Met Ala Gly Arg Asp Leu Gly Asn Ile Leu Thr Ile Leu Ser
            260                 265                 270

ggt agc gag cgg gta cag gcc gcg acg atc aag cgc ttc gcc gac cgc   864
Gly Ser Glu Arg Val Gln Ala Ala Thr Ile Lys Arg Phe Ala Asp Arg
        275                 280                 285
```

```
ttt gct cgc ttc aat ctg cag gag agg gtg aaa gct taa                    903
Phe Ala Arg Phe Asn Leu Gln Glu Arg Val Lys Ala
    290                 295                 300


<210> 6
<211> 300
<212> PRT
<213> Mycobacterium tuberculosis

<400> 6
Asp Tyr Glu Gln Asp Trp Asp Gly Val Ala Ile Thr Leu Thr Arg Ser
  1               5                  10                  15
Gln Leu Tyr Arg Arg Thr Leu Asn Val Ala Gln Glu Leu Ser Arg Cys
            20                  25                  30
Gly Ser Thr Gly Asp Arg Val Val Ile Ser Ala Pro Gln Gly Leu Glu
        35                  40                  45
Tyr Val Val Ala Tyr Leu Gly Ala Leu Gln Ala Gly Arg Ile Ala Val
    50                  55                  60
Pro Leu Ser Val Pro Gln Gly Gly Val Thr Asp Glu Arg Ser Asp Ser
65                  70                  75                  80
Val Leu Ser Asp Ser Ser Pro Val Ala Ile Leu Thr Thr Ser Ser Ala
                85                  90                  95
Val Asp Asp Val Val Gln His Val Ala Arg Arg Pro Gly Glu Ser Pro
            100                 105                 110
Pro Ser Ile Ile Glu Val Asp Leu Leu Asp Leu Asp Ala Pro Asn Gly
        115                 120                 125
Tyr Thr Phe Lys Glu Asp Glu Tyr Pro Ser Thr Ala Tyr Leu Gln Tyr
    130                 135                 140
Thr Ser Gly Ser Thr Arg Thr Pro Ala Gly Val Val Met Ser His Gln
145                 150                 155                 160
Asn Val Arg Val Asn Phe Glu Gln Leu Met Ser Gly Tyr Phe Ala Asp
                165                 170                 175
Thr Asp Gly Ile Pro Pro Pro Asn Ser Ala Leu Val Ser Trp Leu Pro
            180                 185                 190
Phe Tyr His Asp Met Gly Leu Val Ile Gly Ile Cys Ala Pro Ile Leu
        195                 200                 205
Gly Gly Tyr Pro Ala Val Leu Thr Ser Pro Val Ser Phe Leu Gln Arg
    210                 215                 220
Pro Ala Arg Trp Met His Leu Met Ala Ser Asp Phe His Ala Phe Ser
225                 230                 235                 240
Ala Ala Pro Asn Phe Ala Phe Glu Leu Ala Ala Arg Arg Thr Thr Asp
                245                 250                 255
Asp Asp Met Ala Gly Arg Asp Leu Gly Asn Ile Leu Thr Ile Leu Ser
            260                 265                 270
Gly Ser Glu Arg Val Gln Ala Ala Thr Ile Lys Arg Phe Ala Asp Arg
        275                 280                 285
Phe Ala Arg Phe Asn Leu Gln Glu Arg Val Lys Ala
    290                 295                 300


<210> 7
<211> 432
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<221> CDS
<222> (1)..(432)
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<220>
<221> mat_peptide
<222> (1)..(432)

<400> 7

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gcc acc acc ctt ccc gtt cag cgc cac ccg cgg tcc ctc ttc ccc gag   48
Ala Thr Thr Leu Pro Val Gln Arg His Pro Arg Ser Leu Phe Pro Glu
  1               5                  10                  15

ttt tct gag ctg ttc gcg gcc ttc ccg tca ttc gcc gga ctc cgg ccc   96
Phe Ser Glu Leu Phe Ala Ala Phe Pro Ser Phe Ala Gly Leu Arg Pro
            20                  25                  30

acc ttc gac acc cgg ttg atg cgg ctg gaa gac gag atg aaa gag ggg   144
Thr Phe Asp Thr Arg Leu Met Arg Leu Glu Asp Glu Met Lys Glu Gly
        35                  40                  45

cgc tac gag gta cgc gcg gag ctt ccc ggg gtc gac ccc gac aag gac   192
Arg Tyr Glu Val Arg Ala Glu Leu Pro Gly Val Asp Pro Asp Lys Asp
    50                  55                  60

gtc cac att atg gtc cgc gat ggt cag ctg acc atc aag gcc gag cgc   240
Val His Ile Met Val Arg Asp Gly Gln Leu Thr Ile Lys Ala Glu Arg
65                  70                  75                  80

acc gag cag aag gac tta gac ggt cgc tcg gaa ttc gcg tac ggt tcc   288
Thr Glu Gln Lys Asp Leu Asp Gly Arg Ser Glu Phe Ala Tyr Gly Ser
                85                  90                  95

ttc gtt cgc acg gtg tcg ctg ccg gta ggt gct gac gag gac gac att   336
Phe Val Arg Thr Val Ser Leu Pro Val Gly Ala Asp Glu Asp Asp Ile
            100                 105                 110

aag gcc acc tac gac aag ggc att ctt act gtg tcg gtg gcg gtt tcg   384
Lys Ala Thr Tyr Asp Lys Gly Ile Leu Thr Val Ser Val Ala Val Ser
        115                 120                 125

gaa ggg aag cca acc gaa aag cac att cag atc cgg tcc acc aac tga   432
Glu Gly Lys Pro Thr Glu Lys His Ile Gln Ile Arg Ser Thr Asn
    130                 135                 140
```

<210> 8
<211> 143
<212> PRT
<213> Mycobacterium tuberculosis

<400> 8

```
Ala Thr Thr Leu Pro Val Gln Arg His Pro Arg Ser Leu Phe Pro Glu
  1               5                  10                  15
Phe Ser Glu Leu Phe Ala Ala Phe Pro Ser Phe Ala Gly Leu Arg Pro
            20                  25                  30
Thr Phe Asp Thr Arg Leu Met Arg Leu Glu Asp Glu Met Lys Glu Gly
        35                  40                  45
Arg Tyr Glu Val Arg Ala Glu Leu Pro Gly Val Asp Pro Asp Lys Asp
    50                  55                  60
Val His Ile Met Val Arg Asp Gly Gln Leu Thr Ile Lys Ala Glu Arg
65                  70                  75                  80
Thr Glu Gln Lys Asp Leu Asp Gly Arg Ser Glu Phe Ala Tyr Gly Ser
                85                  90                  95
Phe Val Arg Thr Val Ser Leu Pro Val Gly Ala Asp Glu Asp Asp Ile
```

```
                100                 105                 110
Lys Ala Thr Tyr Asp Lys Gly Ile Leu Thr Val Ser Val Ala Val Ser
        115                 120                 125
Glu Gly Lys Pro Thr Glu Lys His Ile Gln Ile Arg Ser Thr Asn
    130                 135                 140
```

<210> 9
<211> 1161
<212> DNA
<213> Mycobacterium tuberculosis

<220>
<221> CDS
<222> (1)..(1161)

<220>
<221> mat_peptide
<222> (1)..(1161)

<400> 9

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aat tcg atg cca gtc ccg ccg gcg cca cca gcg ccg ccg tca ccg atc   48
Asn Ser Met Pro Val Pro Pro Ala Pro Pro Ala Pro Pro Ser Pro Ile
  1               5                  10                  15

aac ccg ccg gtg ccg ccg gtg ccg ccg cta ccg gcc gcg ccc cgg acg   96
Asn Pro Pro Val Pro Pro Val Pro Pro Leu Pro Ala Ala Pro Arg Thr
             20                  25                  30

ctg tcg ccg ccg gta ccg ccg gcg ccg ccg tcg ccg atc agc ttg gcg  144
Leu Ser Pro Pro Val Pro Pro Ala Pro Pro Ser Pro Ile Ser Leu Ala
         35                  40                  45

gcc ccg ccg ctg cca ccg gac ccg ccg atg ccg ccg gcc att tgg tcc  192
Ala Pro Pro Leu Pro Pro Asp Pro Pro Met Pro Pro Ala Ile Trp Ser
     50                  55                  60

gca ctg gag gcg ccg aac cct ccg gtg ccc ccg gcg ccg ccg gga ccg  240
Ala Leu Glu Ala Pro Asn Pro Pro Val Pro Pro Ala Pro Pro Gly Pro
 65                  70                  75                  80

aac agt gcg ccg gca ccg ccg atg ccg ccg acg cct cct ttg ccg ccg  288
Asn Ser Ala Pro Ala Pro Pro Met Pro Pro Thr Pro Pro Leu Pro Pro
                 85                  90                  95

gtg ccg ccg ggg tcg ggc gcg ccg aga ccg gtt ccg gcg gtg ccg cca  336
Val Pro Pro Gly Ser Gly Ala Pro Arg Pro Val Pro Ala Val Pro Pro
            100                 105                 110

atg ccg cca gcg ccg aag agg atg ccg gcg ttg ccg ccc gcc ccg ccg  384
Met Pro Pro Ala Pro Lys Arg Met Pro Ala Leu Pro Pro Ala Pro Pro
        115                 120                 125

gcc ccg ccc tca ccg ccc acg agt tgg tta gcg gtg cca gtt ccg ccg  432
Ala Pro Pro Ser Pro Pro Thr Ser Trp Leu Ala Val Pro Val Pro Pro
    130                 135                 140

gtg ccg ccg gtc ccg ccg ttg ccg gtg aag atg ccg ccg tcg cct ccg  480
Val Pro Pro Val Pro Pro Leu Pro Val Lys Met Pro Pro Ser Pro Pro
145                 150                 155                 160
```

```
gtg ccg ccg ttc cct ccg gcc gag ccg gag act ccg aac ccg ccg gcg   528
Val Pro Pro Phe Pro Pro Ala Glu Pro Glu Thr Pro Asn Pro Pro Ala
                165                 170                 175

ccg ccg gca ccg cca ttg gag aac agc ccg ccg ccc ccg ccg gtg ccg   576
Pro Pro Ala Pro Pro Leu Glu Asn Ser Pro Pro Pro Pro Pro Val Pro
            180                 185                 190

ccg gtg cca ccg gtg ccc ccg ttg acg ctc aac ccg ccg gtg ccg ccg   624
Pro Val Pro Pro Val Pro Pro Leu Thr Leu Asn Pro Pro Val Pro Pro
        195                 200                 205

gca ccg ccg gcg gcc aac acc tcg aac agc ccg ctg cga ccg ccg gcc   672
Ala Pro Pro Ala Ala Asn Thr Ser Asn Ser Pro Leu Arg Pro Pro Ala
    210                 215                 220

ccg ccg gcg cca ccg ttg aag cct ggc ccg ccg gcc ccg ccg atg cca   720
Pro Pro Ala Pro Pro Leu Lys Pro Gly Pro Pro Ala Pro Pro Met Pro
225                 230                 235                 240

ccg gct ccg aac agc ccg gcc gcc ccg ccg tcg ccg ccg agc ccg cct   768
Pro Ala Pro Asn Ser Pro Ala Ala Pro Pro Ser Pro Pro Ser Pro Pro
                245                 250                 255

gtg ccg gtg ttc ccg act ccg ccg ggc ccc ccg gcg ccg ccg gag ccg   816
Val Pro Val Phe Pro Thr Pro Pro Gly Pro Pro Ala Pro Pro Glu Pro
            260                 265                 270

aac agc agc ccg ccg gcc ccg ccg gcc ccg cca gcc gcg ccg ttg ccc   864
Asn Ser Ser Pro Pro Ala Pro Pro Ala Pro Pro Ala Ala Pro Leu Pro
        275                 280                 285

ggg ccg tca ccc ccg gcc cca ccc gcc ccg ccg ttg ccg aat agc ccc   912
Gly Pro Ser Pro Pro Ala Pro Pro Ala Pro Pro Leu Pro Asn Ser Pro
    290                 295                 300

gcg gct cca cct ggg ccg ccg gcc tgg ccg ggc gcc ccg gac ccg ccg   960
Ala Ala Pro Pro Gly Pro Pro Ala Trp Pro Gly Ala Pro Asp Pro Pro
305                 310                 315                 320

gcc ccg ccg ttg ccg tac agc agc ccg ccg gcc ccg ccg gct tgc ccg  1008
Ala Pro Pro Leu Pro Tyr Ser Ser Pro Pro Ala Pro Pro Ala Cys Pro
                325                 330                 335

gtc ccc ggt gcg ccg ttg gcg ccg ttg ccg atc agc gga cgc ccc agc  1056
Val Pro Gly Ala Pro Leu Ala Pro Leu Pro Ile Ser Gly Arg Pro Ser
            340                 345                 350

aac agc tgg gtg ggc gtg ttc aca atg ttg agc agg ccc tcc aac ggc  1104
Asn Ser Trp Val Gly Val Phe Thr Met Leu Ser Arg Pro Ser Asn Gly
        355                 360                 365

gcc gcg gcg gcg gcc tca gcg ctc gcg tac gcg cca gcg ccc gca gta  1152
Ala Ala Ala Ala Ala Ser Ala Leu Ala Tyr Ala Pro Ala Pro Ala Val
    370                 375                 380

aag gtt tga                                                     1161
Lys Val
385
```

<210> 10
<211> 386
<212> PRT
<213> Mycobacterium tuberculosis

<400> 10

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Asn Ser Met Pro Val Pro Pro Ala Pro Pro Ala Pro Pro Ser Pro Ile
 1               5                  10                  15
Asn Pro Pro Val Pro Pro Val Pro Pro Leu Pro Ala Ala Pro Arg Thr
            20                  25                  30
Leu Ser Pro Pro Val Pro Pro Ala Pro Pro Ser Pro Ile Ser Leu Ala
        35                  40                  45
Ala Pro Pro Leu Pro Pro Asp Pro Pro Met Pro Pro Ala Ile Trp Ser
    50                  55                  60
Ala Leu Glu Ala Pro Asn Pro Pro Val Pro Pro Ala Pro Pro Gly Pro
65                  70                  75                  80
Asn Ser Ala Pro Ala Pro Pro Met Pro Pro Thr Pro Pro Leu Pro Pro
                85                  90                  95
Val Pro Pro Gly Ser Gly Ala Pro Arg Pro Val Pro Ala Val Pro Pro
            100                 105                 110
Met Pro Pro Ala Pro Lys Arg Met Pro Ala Leu Pro Pro Ala Pro Pro
        115                 120                 125
Ala Pro Pro Ser Pro Pro Thr Ser Trp Leu Ala Val Pro Val Pro Pro
    130                 135                 140
Val Pro Pro Val Pro Pro Leu Pro Val Lys Met Pro Pro Ser Pro Pro
145                 150                 155                 160
Val Pro Pro Phe Pro Pro Ala Glu Pro Glu Thr Pro Asn Pro Pro Ala
                165                 170                 175
Pro Pro Ala Pro Pro Leu Glu Asn Ser Pro Pro Pro Pro Pro Val Pro
            180                 185                 190
Pro Val Pro Pro Val Pro Pro Leu Thr Leu Asn Pro Pro Val Pro Pro
        195                 200                 205
Ala Pro Pro Ala Ala Asn Thr Ser Asn Ser Pro Leu Arg Pro Pro Ala
    210                 215                 220
Pro Pro Ala Pro Pro Leu Lys Pro Gly Pro Pro Ala Pro Pro Met Pro
225                 230                 235                 240
Pro Ala Pro Asn Ser Pro Ala Ala Pro Pro Ser Pro Pro Ser Pro Pro
                245                 250                 255
Val Pro Val Phe Pro Thr Pro Pro Gly Pro Pro Ala Pro Pro Glu Pro
            260                 265                 270
Asn Ser Ser Pro Pro Ala Pro Pro Ala Pro Pro Ala Ala Pro Leu Pro
        275                 280                 285
Gly Pro Ser Pro Pro Ala Pro Pro Ala Pro Pro Leu Pro Asn Ser Pro
    290                 295                 300
Ala Ala Pro Pro Gly Pro Pro Ala Trp Pro Gly Ala Pro Asp Pro Pro
305                 310                 315                 320
Ala Pro Pro Leu Pro Tyr Ser Ser Pro Pro Ala Pro Pro Ala Cys Pro
                325                 330                 335
Val Pro Gly Ala Pro Leu Ala Pro Leu Pro Ile Ser Gly Arg Pro Ser
            340                 345                 350
Asn Ser Trp Val Gly Val Phe Thr Met Leu Ser Arg Pro Ser Asn Gly
        355                 360                 365
Ala Ala Ala Ala Ala Ser Ala Leu Ala Tyr Ala Pro Ala Pro Ala Val
    370                 375                 380
Lys Val
385
```

CLAIMS:

1. An isolated polypeptide or a derivative, homologue, analogue or functional equivalent thereof wherein said polypeptide is obtainable from a species of *Mycobacterium* and which polypeptide is immunointeractive with sera from a human, animal or avian species exposed to said species of *Mycobacterium* or its relative or antigenic parts thereof but which polypeptide is substantially not immunointeractive with sera from a human, animal or avian species not prior exposed to said species of *Mycobacterium* or its relative or its antigenic parts.

2. An isolated polypeptide according to claim 1 wherein the species of *Mycobacterium* is selected from *Mycobacterium* is *M. tuberculosis, Mycobacterium avium, Mycobacterium microti, Mycobacterium leprae, Mycobacterium lepraemurium, Mycobacteria paratuberculosis, Mycobacterium ulcerans, Mycobacterium marinum, Mycobacterium smegmatis, Mycobacterium intracellulare, Mycobacterium xenopi, Mycobacterium chelonei, Mycobacterium fortuitum, Mycobacterium farcinogenes, Mycobacterium flavum, Mycobacterium haemophitum, Mycobacterium kansasii, Mycobacterium phlei, Mycobacterium scrofulaceum, Mycobacterium senegalense, Mycobacterium simiae, Mycobacterium thermoresistible,* and *Mycobacterium xenopi.*

3. An isolated polypeptide according to claim 2 wherein the species of *Mycobacterium* is *M. tuberculosis.*

4. An isolated polypeptide or a derivative, homologue, analogue or functional equivalent thereof wherein said polypeptide is obtainable from *M. tuberculosis* or a related organism and which polypeptide is immunointeractive with sera from a human previously exposed to *M. tuberculosis* or an antigenic extract therefrom but is substantially not immunointeractive with human sera not previously exposed to *M. tuberculosis* or a antigenic extract thereof.

5. An isolated polypeptide according to claim 4 wherein the human exposed to *M. tuberculosis* has active pulmonary or extra-pulmonary tuberculosis.

6. An isolated polypeptide according to claim 4 or 5 wherein the polypeptide has a molecule weight of from about 5 kDa to about 100 kDa.

7. An isolated polypeptide according to claim 6 wherein the molecular weight is selected from about 10 to 20 kDa, 28 to 38 kDa, 38 to 48 kDa, 53 to 63 kDa and 55 to 65 kDa.

8. An isolated polypeptide comprising an amino acid sequence selected from <400>2, <400>4, <400>6, <400>8, <400>10 or an amino acid sequence having at least 60% similarity to any one of said sequences.

9. An isolated polypeptide encoded by a nucleotide sequence selected from <400>1, <400>3, <400>5, <400>7, <400>9 or an nucleotide sequence having at least 60% similarity to any one of said sequences or a nucleotide sequence capable of hybridizing to any one of said sequences under low stringency conditions at 42°C.

10. A method of isolating a polypeptide from *Mycobacterium* species said method comprising culturing cells of said *Mycobacterium* species in a growth medium to increase the number of cells to a sufficient population, harvesting said cells and subjecting said cells to protein extraction techniques to extract protein from said cells, fractionating the extracted protein and subjecting said protein to binding analysis with antibodies to said *Mycobacterium* species or antigenic portions thereof and isolating the polypeptides to which antibodies interact.

11. A method according to claim 10 wherein the *Mycobacterium* species is *M. tuberculosis.*

12. A method according to claim 10 or 11 wherein the growth medium is Lowensten-Jensen medium.

13. A method according to claim 10 wherein the binding analysis is conducted by Western blotting and other immunoassay procedures.

14. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a polypeptide obtainable from a species of *Mycobacterium* and which polypeptide is immunointeractive with sera from a human, animal or avian species exposed to said species of *Mycobacterium* or its relative or antigenic parts thereof but which polypeptide is substantially not immunointeractive with sera from a human, animal or avian species not prior exposed to said species of *Mycobacterium* or its relative or its antigenic parts.

15. An isolated nucleic acid molecule according to claim 14 wherein the *Mycobacterium* species is *M. tuberculosis.*

16. An isolated nucleic acid molecule according to claim 15 wherein the nucleotide

sequence encodes an amino acid sequence substantially as set forth in <400>1, <400>3, <400>5, <400>7, <400>9 or an amino acid sequence having at least 60% similarity thereto.

17. An isolated nucleic acid molecule according to claim 15 wherein the nucleotide sequence is substantially as set forth in <400>1, <400>3, <400>5, <400>7, <400>9 or an nucleotide sequence having at least 60% similarity to any one of said sequences or a nucleotide sequence capable of hybridizing to any one of said sequences under low stringency conditions at 42°C.

18. An antibody specific to the polypeptide according to any one of claims 1 to 9.

19. An antibody according to claim 18 wherein the antibody is a monoclonal antibody.

20. A method for detecting the presence of *M. tuberculosis* such as in a patient suffering from tuberculosis said method comprising contacting a biological sample from a patient or subject with an antibody specific for a polypeptide from said *M. tuberculosis* and detecting a complex between said polypeptide and said antibody.

21. An assay device for *M. tuberculosis* comprising a solid support having immobilized thereon one or more polypeptides obtainable from *M. tuberculosis* or derivatives, homologues, analogues or antigenic equivalents thereof and a portion of said solid support adapted for receiving a sample from a human subject to be tested wherein said sample would contain an antibody specific for said *M. tuberculosis* polypeptide if said subject has been exposed to *M. tuberculosis* wherein upon contact between the antibody from the subject and the immobilized polypeptide, a complex forms and said complex is detected by an anti-human immunoglobulin labelled with a reporter molecule.

22. Use of a polypeptide according to any one of claims 1 to 9 in the manufacture of a medicament for the treatment of *Mycobacterium* infection in a human, animal or bird.

23. Use according to claim 22, wherein the *Mycobacterium* is *M. tuberculosis* and the infection in human.

24. A method for treating or preventing infection by *Mycobacteria* species in a human, animal or avian species said method comprising administering to said human, animal or avian species an immune response stimulating an effective amount of a polypeptide according to any one of claims 1 to 9.

25. A method according to claim 24 wherein the species of *Mycobacterium* is *M. tuberculosis* and the infection is in humans.

26. A method according to claim 24 wherein the infection is active pulmonary tuberculosis or extra-pulmonary tuberculosis.

ABSTRACT

The present invention relates generally to molecules derived from a *Mycobacterium* species and recombinant, synthetic, derivative, homologue and analogue forms of said molecules. The molecules of the present invention are useful in diagnostic assays for *Mycobacterium* in biological and environmental samples. The present invention is particularly directed to molecules derived from *Mycobacterium tuberculosis* and related organisms and even more particularly to recombinant forms of these molecules or synthetic, derivative, homologue or analogue forms thereof and their use in diagnostic and therapeutic protocols for tuberculosis or other disease conditions associated with *M. tuberculosis* or related organisms.

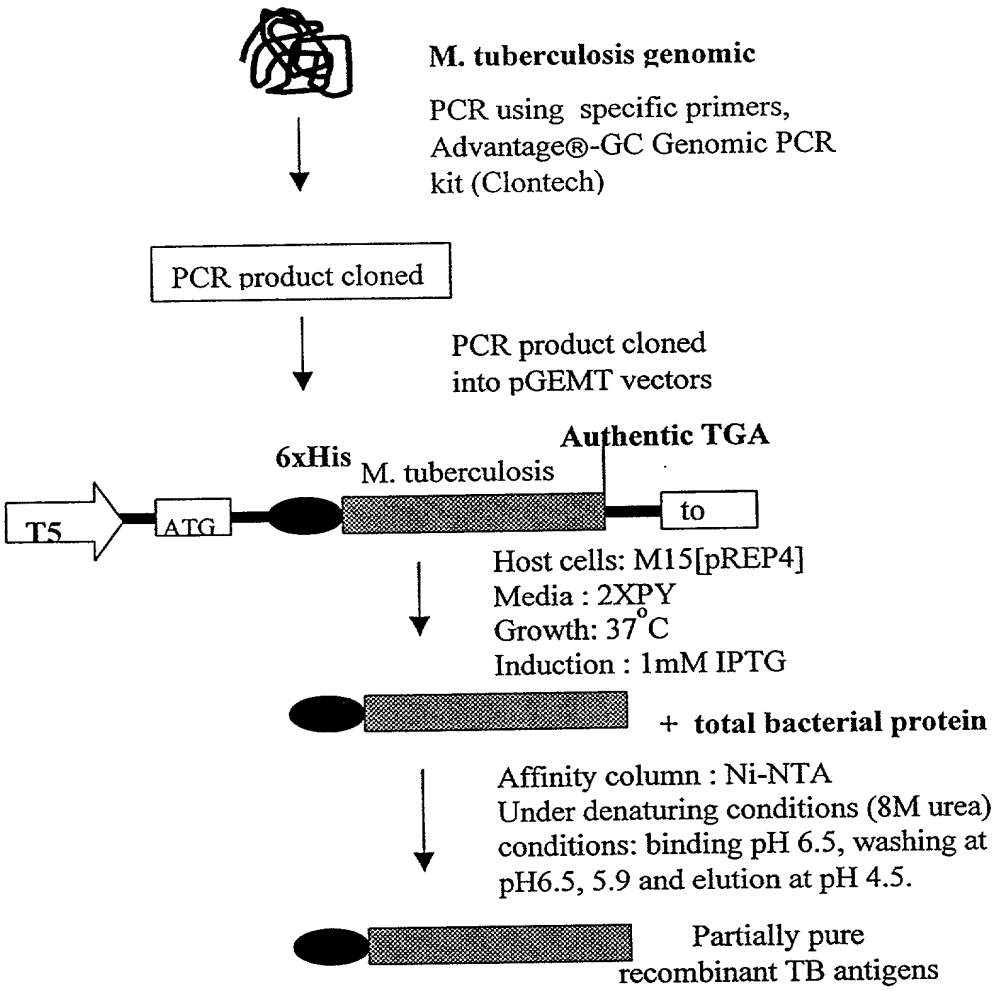
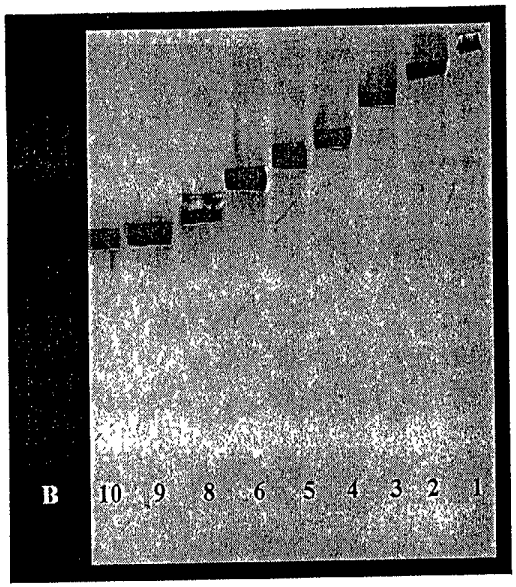
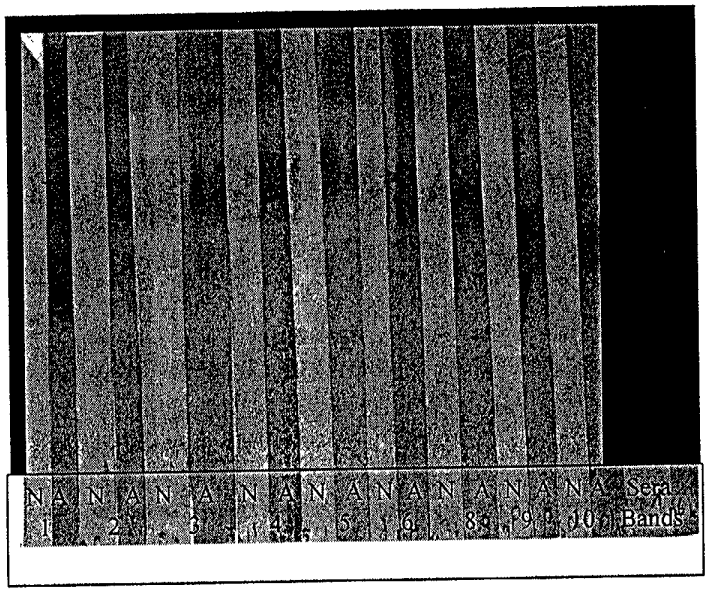


Fig.1 Strategy for the isolation and expression of M.tuberculosis protein antigens.



(A)



(B)

Fig.2. (A) Gel purified and concentrated M. tuberculosis protein bands (B.1, 2, 3, 4, 5, 6, 8, 9, 10) blotted onto PVDF membrane were excised for N-terminal sequencing. (B) Concentrated M. tuberculosis protein bands blotted onto nitrocellulose membrane and immuno-screened using pooled normal (N) and active (A) sera respectively. Positive bands (arrows) were observed with A but not with N.

Fig. 3 Result of homology search against the GenBank protein sequence databases. Proteins showing the highest homology to the M. tuberculosis protein bands are as shown.

Relative molecular weight (kDa)	**Sequence from N-terminal sequencing**	**Match (GenBank)**
B.4	SKLIEYDELALEAME	db: $_{2}$SKLIEYDETARHAME$_{16}$ 55.74kDa, groEL1/protein cpn60 [16], pID=g44601, X60350 (80% match)
B.5	AKTIAYDEEARV	db: $_{2}$AKTIAYDEEA$_{10}$ 56.728 kDa, CHAPERONIN2, groEL2, GenBank pID=g15000, MTTCWPA_3 (100% match)
B.6	AEVDAYKFDPDAVD	db: $_{161}$AEFDAYRRDPMA$_{172}$ Probable exported protease, has signal sequence, very similar to three proteases / peptidases from Streptomyces, pID=e235164, MTCY427.04c (51% match)
B.9	AEYTLPDLDWDYG	db: $_{2}$AEYTLPDLDWDYG$_{14}$ 23.0 kDa, superoxide dismutase, pID=g581379, MTSOD4 (100% match)
B.10	MEIDILAVAAP	db: $_{117}$IEVDLLDLDAP$_{127}$ 33 kDa, mycocerosic acid synthase [17], pID=g149978, M95808 (56.9% match)
MMP	ATTLPVQRHDARL	db:ATLPVQRHPRSL 14/16 kDa [18], pID=g244562, M76712

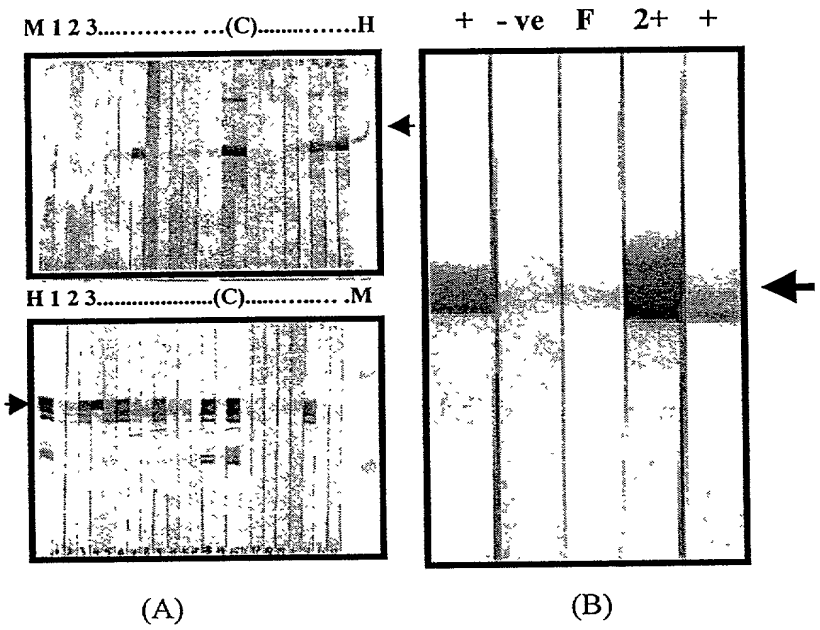
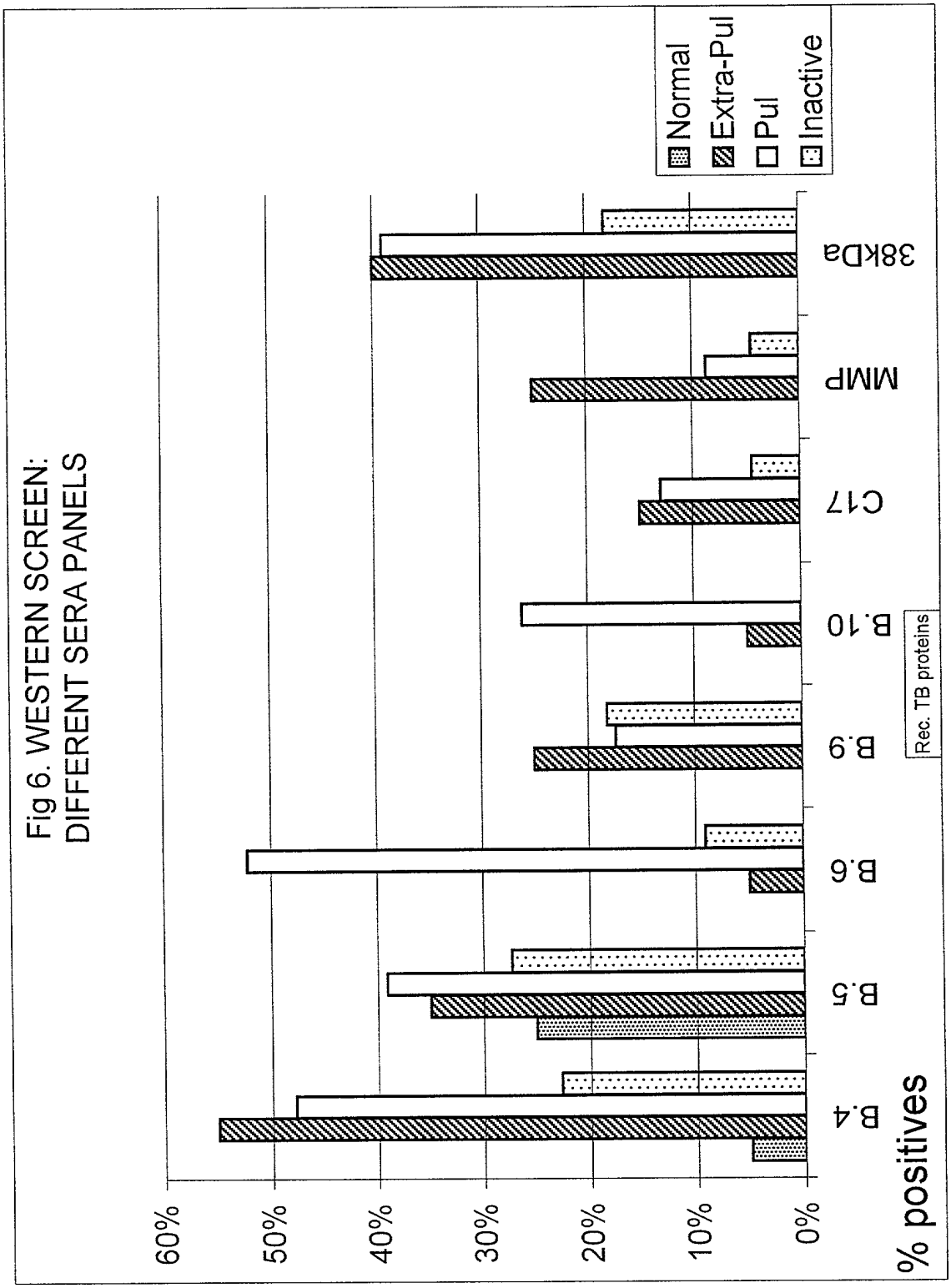


Fig. 4. Western screening of recombinant M. tuberculosis antigens. (A) Arrows indicate the position of the recombinant antigens on the membrane. M= Kaleidoscope protein Marker and H= strip probed with anti-RGSHis, C= a positive control of strips probed with known human serum reactive to the specific recombinant antigen. (B) Reactivity is estimated based on the intensity of band on

Fig 5. Percentage of reactivity of recombinant TB antigens against different sera panels. A known 38kDa antigen [20, 21] of M. tuberculosis was included in the screening. The gene (GeneBank Accession # M30046) for this antigen was cloned, expressed in pQE30 and partially purified as described in section E. Also shown are the percentage of reactivity of sera samples detected by a commercially available rapid TB diagnostic kit from ICT (Amrad).

Sera Panel: Recombinant antigens:	Uninfected (normal)	Active TB (Extra-Pulmonary)	Active TB (Pulmonary)	Inactive
B.4	5%	55%	47.8%	22.7%
B.5	25%	35%	39.1%	27.3%
B.6	0%	5%	52.2%	9.1%
B.9	0%	25%	17.4%	18.2%
B.10	0%	5%	26.1%	0%
MMP	0%	25%	8.7%	4.5%
C17	0%	15%	13.0%	4.5%
38 kDa	0%	40%	39.1%	18.2%
ICT TB Kit	0%	55%	52.2%	13.6%

Fig 6. WESTERN SCREEN:
DIFFERENT SERA PANELS



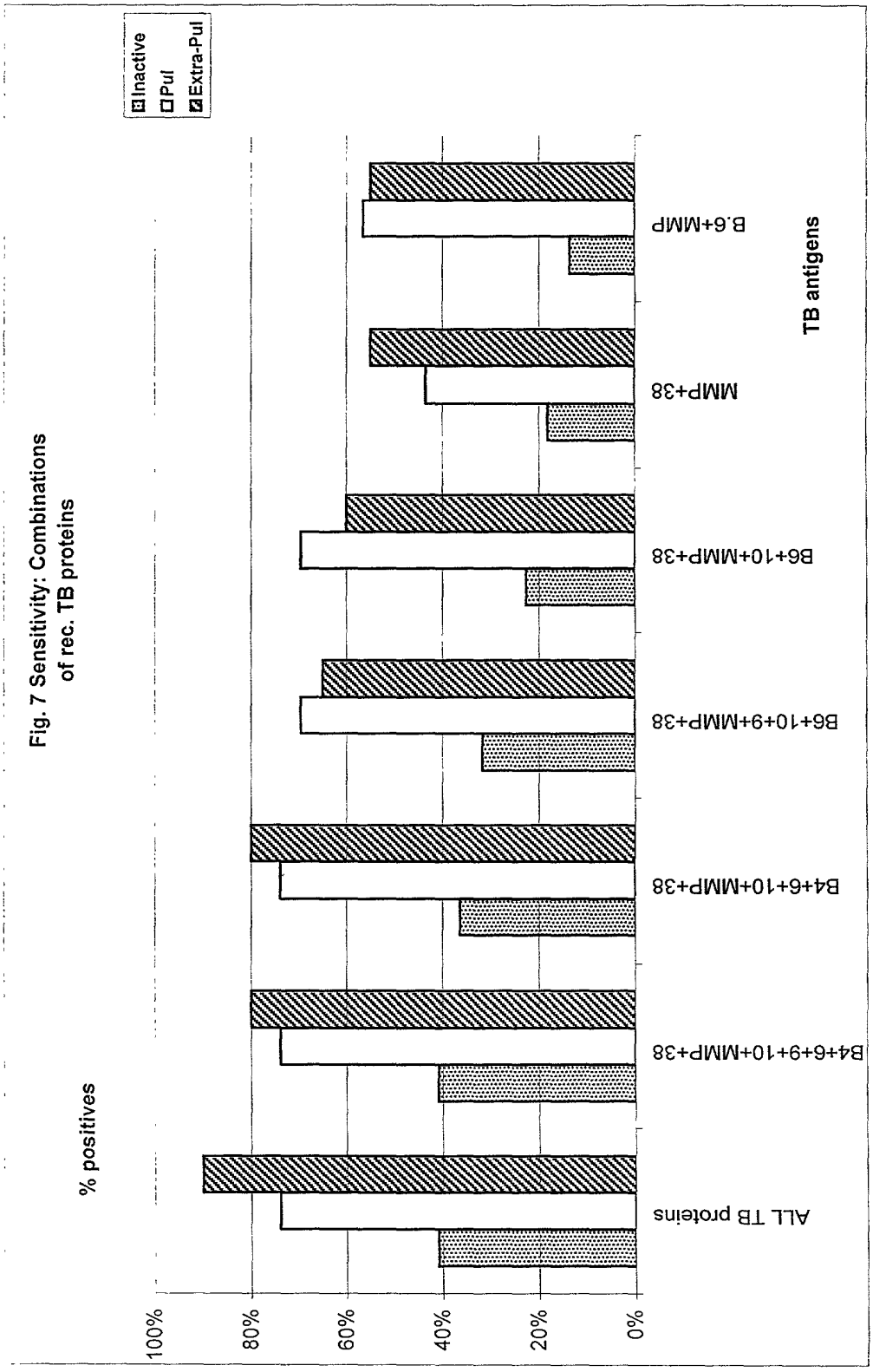
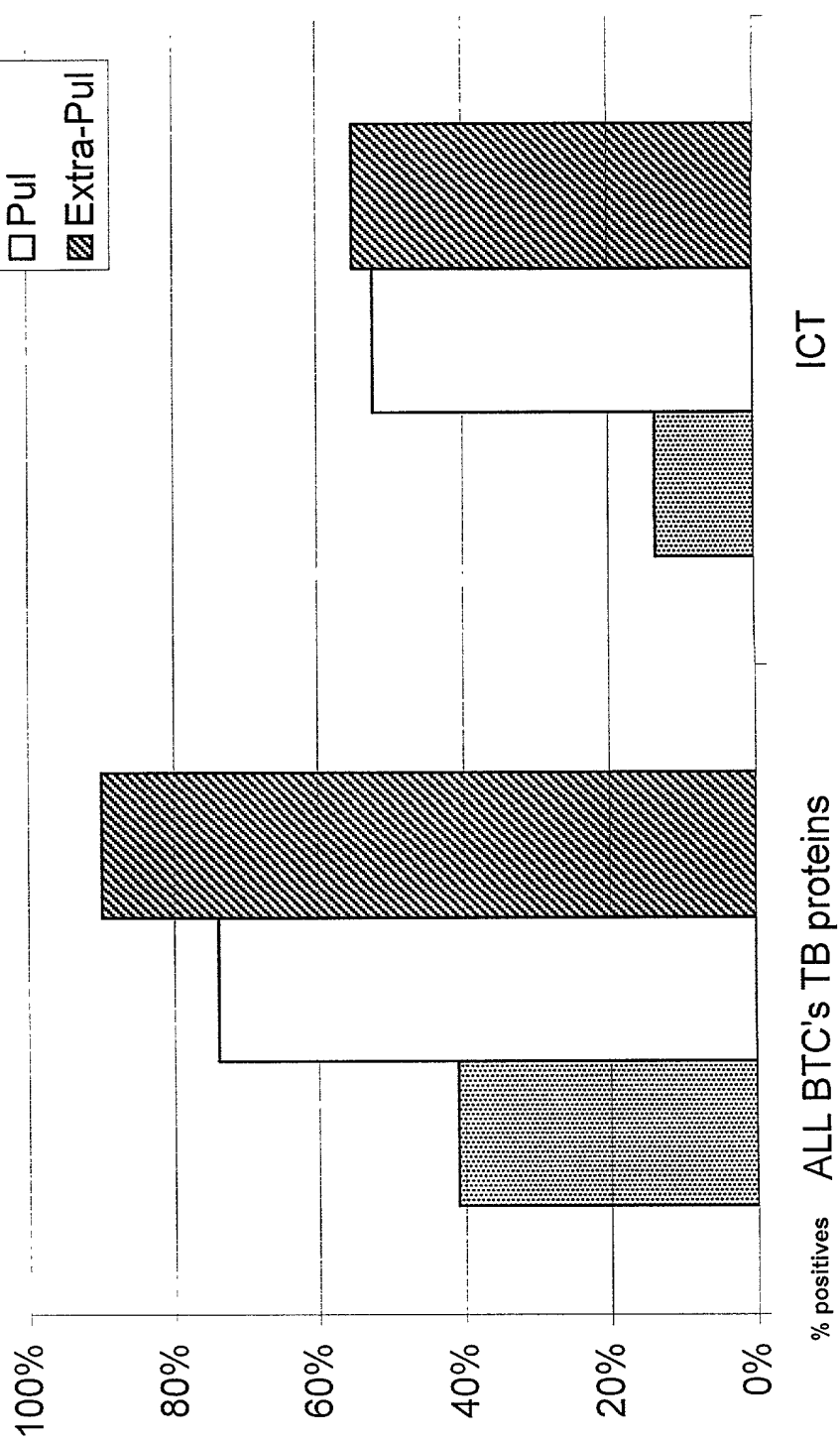


Fig. 7 Sensitivity: Combinations of rec. TB proteins

Fig. 8 Comparison of our rec. TB proteins with the ICT TB diagnostic kit



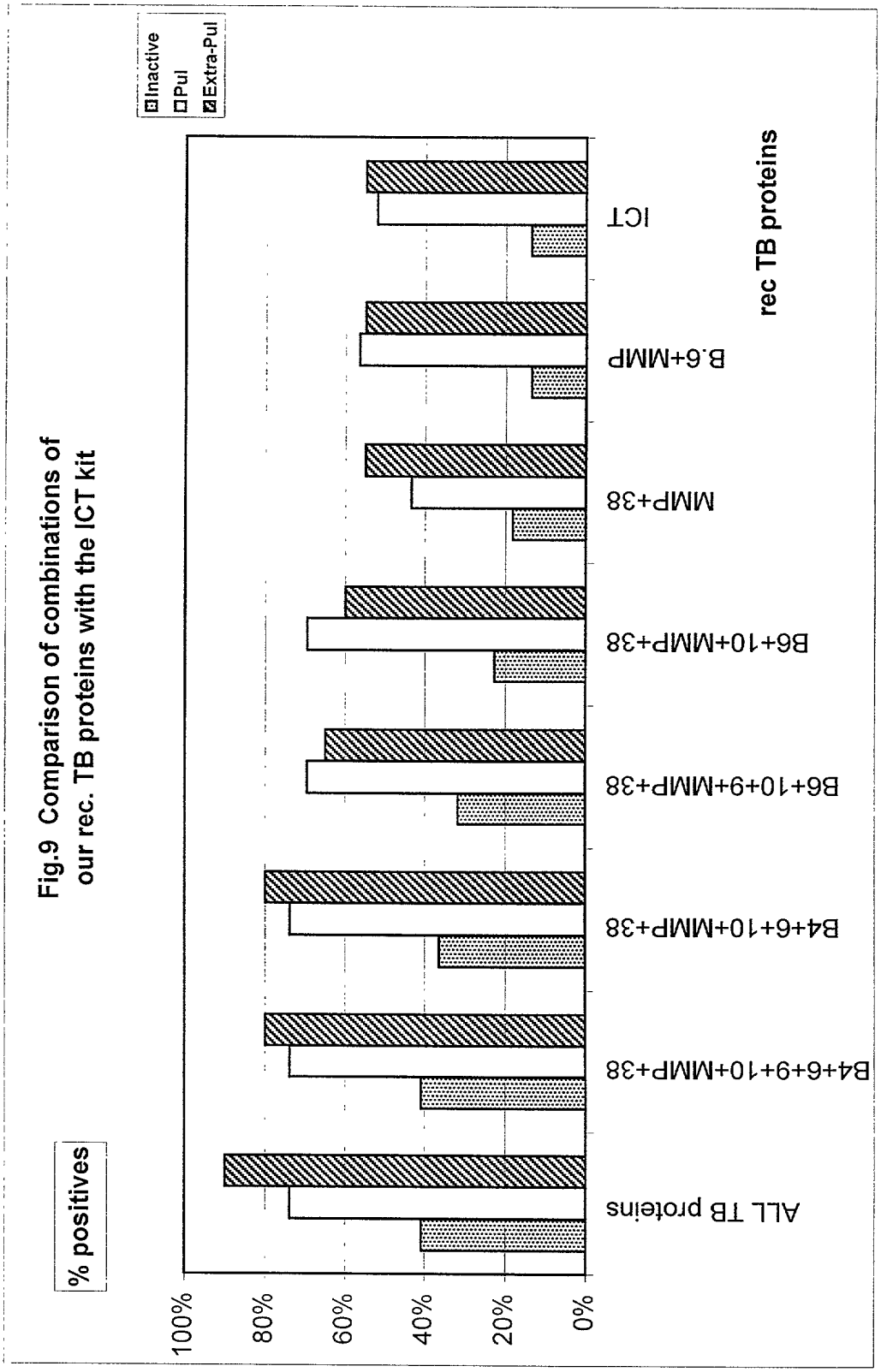


Fig.9 Comparison of combinations of our rec. TB proteins with the ICT kit

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COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that. my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Insert Title: BACTERIAL-DERIVED MOLECULES AND THERAPEUTIC AND DIAGNOSTIC USES THEREFOR

Fill in Appropriate Information - For Use Without Specification Attached:

the specification of which is attached hereto. If not attached hereto,
the specification was filed on _____ as
United States Application Number _____;
and amended on _____ (if applicable) and/or
the specification was filed on _____ as PCT
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I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows

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Insert Priority Information: (if appropriate)

Prior Foreign Application(s)			Priority Claimed	
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No

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Insert Prior U.S. Application(s): (if any)

(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)
60/112,499	December 16, 1998	Pending

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary

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